EVALUATION OF CLINICAL EFFECTIVENESS OF PLATELET RICH FIBRIN AND BONE GRAFT IN MANAGEMENT OF INTRABONY DEFECTS : A COMPARATIVE STUDY

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partial fulfillment of the requirements
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MASTER OF DENTAL SURGERY

BRANCH – II
PERIODONTICS

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
Chennai – 600 032

2010 - 2013

CERTIFICATE

This is to certify that **Dr. SHRUTI BERI**, Post Graduate student (2010-2013) in the Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai - 600 003, has done this dissertation titled **"EVALUATION OF CLINICAL EFFECTIVENESS OF PLATELET RICH FIBRIN AND BONE GRAFT IN MANAGEMENT OF INTRABONY DEFECTS : A COMPARATIVE STUDY "** under the direct guidance and supervision in partial fulfillment of the regulations laid down by the **Tamil Nadu Dr. M.G.R. Medical University**, Chennai - 600 032 for **M.D.S.,** (Branch-II) **Periodontics** degree examination.

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ABSTRACT

Background : Periodontal therapy aims to prevent periodontal tissue destruction while achieving regeneration of lost and damaged tissues. Demineralized bone matrix (DMBM) is a xenograft with acceptable clinical responses in the field of periodontal regeneration. Platelet Rich Fibrin (PRF) is the latest advancement in fibrin technology and is a rich autologous source of various growth factors and leukocytes. PRF has a strong potential to influence the cellular mechanisms responsible for periodontal regeneration to be achieved. A combination of the two grafting modalities may prove to be an advantageous regenerative treatment option for management of intrabony defects.

Aim: The aim of this study was to clinically and radiographically evaluate, the additional effectiveness of autologous PRF, when used in combination with bone graft (DMBM) as compared to bone graft (DMBM) alone, in the treatment of intrabony defects.

Methods : A total of 18 intrabony defects in 15 systemically healthy patients were selected randomly for the purpose of the study. The defects were equally divided into two groups and treated with DMBM alone and in combination with PRF. Clinical parameters such as plaque index (PI), gingival bleeding index (GBI), probing pocket depth (PPD), and clinical attachment level (CAL) were recorded at baseline and at 6 months post-operatively. In both the groups, radiographic analysis was performed at baseline, and at 3 months and 6 months post operatively.

Results :. Significant reduction in post- operative mean pocket depth and gain in attachment level was observed in PRF-DMBM and DMBM groups as compared to baseline. Also, greater attachment gain was observed in PRF-DMBM group post-operatively. Radiographically, at the end of 6 months, reduction in defect depth was significantly greater in PRF-DMBM group than DMBM group ($p=0.007$). Greater gain in percentage of bone fill was observed for PRF-DMBM group than for DMBM group at 6 months. Percentage of original defect resolution was statistically greater in PRF-DMBM group (40.23 ±29.41 %) as compared to DMBM alone (12.43±14.14 %) at 3 months.

Conclusion : Autologous PRF when added to DMBM demonstrated additional effectiveness and ability to augment the effects of bone graft material, clinically and radiographically in management of periodontal intrabony defects.

Keywords : Demineralized bone matrix, Intrabony defect, Periodontal Regeneration, Platelet Rich Fibrin.

DECLARATION

TITLE OF DISSERTATION	Evaluation of clinical effectiveness of platelet rich fibrin and bone graft in management of intrabony defects : A comparative study
PLACE OF STUDY	Tamil Nadu Government Dental College & Hospital, Chennai-600003
DURATION OF THE COURSE	3 Years
NAME OF THE GUIDE	Dr. K. Malathi
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Mrs. Dr. K. MALATHI aged 46 years working as **Professor & H.O.D.** in Department of Periodontics at the college, having residence address at 10/221, Subamathi Nivas, Plot No 96, 4th Cross street, Vimala Nagar, Medavakkam, Chennai - 100 (herein after referred to as the 'Principal Investigator')

And

Mrs. Dr. SHRUTI BERI aged 27 years currently studying as final year **Post graduate student** in the Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai -3 (herein after referred to as the 'PG and co-investigator') residing at D-10/A , SFS Flats, Mayur Vihar Phase III, Delhi 110096.

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R.C.No. 0430/DE/2010 Date: 09.08.2011

Title of the Work : Evaluation of clinical effectiveness of Platelet Rich Fibrin and bone graft in management of intrabon defects: A compative study

Principal Investigator: Dr.Shruti Beri, IInd Year MDS., PG student,

Department : Dept. of Periodontics
Tamil Nadu Govt Dental College and Hospital, Chennai-3

The request for an approval from the Institutional Ethical Committee (IEC) was considered for the following on the IEC meeting held on 25.07.2011 at the Principal's Chambers, Tamil Nadu Government Dental College & Hospital, Chennai-3.

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Tamil Nadu Government Dental College and Hospital, Chennai-3

Telephone No: 044 2534 0343
Fax : 044 2530 0681

R.C.No. 0430/DE/2010 Date: 07-03-2012

Title of the Work	**"Evaluation of clinical effectiveness of Patelet Rich Fibrin and bone graft in management of intrabony defects: A Comparative Study"**
Principal Investigator:	Dr. Shruti Beri, P.G. II Year Student
Department	Department of Periodontics

The request for an approval from the Institutional Ethical Committee (IEC) was considered for the following on the **IEC meeting held on 25-01-2012** at the Principal's Chambers, Tamil Nadu Government Dental College & Hospital, Chennai-3.

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LIST OF ABBREVIATIONS

ABBM	Anorganic Bovine Bone Mineral
AC	Alveolar Crest
BCC	Bone Crest Change
BD	Base of the defect
BDX	Bovine Derived Xenograft
BF	Bone Fill
BL	Baseline
BMP	Bone Morphogenic Protein
BMSCs	Bone Marrow Stromal Cells
BOP	Bleeding On Probing
BPBM	Bovine Porous Bone Mineral
CAL	Clinical Attachment Level
CEJ	Cemento-Enamel Junction
CF	Correction Factor
CIF	Cartilage Inducing Factor
DBM	Demineralized Bone Matrix
DD	Defect Depth
DFDBA	Demineralized Freeze Dried Bone Allograft
DMBM	Bovine Derived Demineralized Bone Matrix
DR	Defect Resolution
EGF	Epidermal Growth Factor
FDBA	Freeze Dried Bone Allograft
FDP	Fibrin Degradation Products
GBI	Gingival Bleeding Index
GF	Growth Factors
GTR	Guided Tissue Regeneration
HA	Hydroxyapatite
HCl	Hydrochloride

IGF	Insulin like growth factor
IL	Interleukins
IOPA	Intra Oral Peri-apical
NS	Non significant
P value	Probability value
PDGF	Platelet Derived Growth Factor
PI	Plaque Index
PPD	Pocket Probing Depth
PPP	Platelet Poor Plasma
PRF	Platelet Rich Fibrin
PRP	Platelet Rich Plasma
RBC	Red Blood Cell
S	Significant
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
RA	Root Apex
TGF β	Transforming Growth Factor β
VEGF	Vascular Endothelial Growth Factor

INTRODUCTION

The goal of periodontal therapy includes arrest of periodontal disease progression and the regeneration of structures lost due to pre-existing disease process. Conventional surgical techniques offer only limited potential towards recovering the lost periodontal structures.

Successful periodontal reconstruction comprises of regeneration of multiple tissues of the periodontium. It is a complex biological process in itself which is intricately regulated between cells, locally acting growth factors and the extracellular matrix components. The key to periodontal regeneration is to stimulate the progenitor cells to re-occupy the defect [44].

Earlier attempts to achieve regeneration included denudation of interdental bone to treat intrabony defects and use of autografts to fill the surgical site. Also, favourable results have been gained in treatment of such defects using a combination of graft material and collagen membranes.[84]

However, recently, the attention has shifted to the use of growth factors which are the biologic mediators that can regulate the proliferation, chemotaxis and differentiation of the locally derived progenitor cells in the defect site.[17]

Among the rich sources of autologous growth factors the various generations of platelet concentrates are currently in use. Platelet Rich Plasma, first generation concentrate, has been used alone and in combination with grafting materials and barrier membranes in treatment of periodontal and surgical defects.[3,13,35] However, the effects of Platelet rich plasma on bone regeneration have been limited.

The second and latest generation of platelet concentrates is Platelet Rich Fibrin. It is a promising, completely autologous leukocyte and platelet concentrate which is being successfully used in various fields of dentistry and medicine. PRF has shown successful results when used as a sole agent in the treatment of periodontal intrabony defects [100]. However, limited research is available for PRF as a combination therapy with bone graft materials.[62, 78]

It remains to be evaluated how well a combination of commercially available grafts and autologous growth factors can alter or enhance the potential for regeneration.

Hence, the present study has been undertaken to evaluate and compare clinical and radiographic effectiveness of combination of platelet rich fibrin with bone graft in relation to bone graft alone.

AIM

The aim of the present study is to investigate the **additional effectiveness** of autologous PRF with bone graft (DMBM) in the treatment of three wall intrabony osseous defects in comparison with bone grafts alone (DMBM) .

OBJECTIVES

The objectives of the study included :

1. To **clinically** evaluate and compare the use of PRF and bone graft (DMBM) in the management of intrabony osseous defects.

2. To **radiographically** compare and assess the regeneration of lost alveolar bone by PRF and bone graft (DMBM) in the management of intrabony osseous defects.

REVIEW OF LITERATURE

1. REGENERATION OF PERIODONTAL TISSUES

Periodontitis is an inflammatory disease characterized by destruction of alveolar bone, root cementum, periodontal ligament and gingiva as a response to insults elicited by microbial accumulations on tooth surfaces.[59] These responses can result in variety of intraosseous defects of various architectures.

Periodontal regeneration refers to complete restoration of functional supporting tissues, including alveolar bone, cementum and periodontal ligament. It is defined as the reproduction or reconstruction of lost or injured part with form and function of lost structures restored.[2]

Melcher et al[70] in ***1976*** proposed the type specific repopulation theory, which was further established by ***Gotlow et al***[44] in **1986**. The theory states that, different periodontal connective tissues compete for the root surface during healing each resulting in a selected cell population occupying the periodontal wound and resulting in a specific type of repair or regeneration.

Trombelli et al[104] in ***2002***, in their systematic review reported various grafting modalities and bone substitutes that have been in use over the years for regenerative purposes. They compared results of open flap debridement alone and in combination with graft materials and concluded implantation of graft materials provided favourable results such as gain in clinical attachment levels, reduction in pocket probing depths and gain in defect fills.

Needleman[74] in ***2002*** and ***Giannobile & Somerman***[42] in ***2003***, in their respective systematic reviews on application of guided tissue regeneration and enamel

matrix derivatives reported significant increase in clinical attachment levels (CAL), however the magnitude of the observed additional benefits were modest.

Although, periodontal regeneration is a possible objective of several periodontal therapeutic modalities, outcomes of such modalities are not always predictable. Complete regeneration may be an unrealistic goal for many situations due in part to the complexity of the biological events and cells underlying successful periodontal regeneration.[84]

Wang et al[106] *in* ***2006*** concluded that various factors which determine the predictability of bone regeneration include primary wound closure, blood supply, defect architecture, space maintenance and wound stability. All these factors play a significant role in deciding the amount and extent of achievable regeneration via various grafting modalities.

2. BONE GRAFTS

Regeneration of lost bone and periodontal attachment apparatus can improve the health of supporting periodontal tissues. Bone replacement grafts have been used to facilitate and promote this periodontal regeneration.

As early as ***1923 Hegedus***[49] reported rebuilding of the alveolar process by bone transplantation. In ***1971, Shaffer***[93] used plaster of paris as one of the first synthetic implant material used in periodontics. Since then, bone replacement graft materials have fascinated various surgeons for their application and successful outcomes in treatment of bony defects.

To achieve successful regeneration any substitute should be biologically compatible, non-toxic and provide scaffolding for angiogenesis and new bone outgrowth. A graft material must possess either osteogenic, osteoinductive or

osteoconductive activity. Broadly, bone grafts are classified as autografts, allografts, xenografts and alloplasts.

Froum et al[37] *in* ***1976*** clinically evaluated and compared responses of human periodontal defects following open debridement with and without the subsequent implantation of an osseous coagulum-bone blend graft and reported greater levels of osseous regeneration with the autogenous graft procedures than following open debridement alone.

Petite et al[76] *in* ***2000***, concluded that autogenous bone grafts are the preferred choice for any regenerative procedure, however patient morbidity, limited supply of suitable bone, painful procurement, risk of infection, nerve damage and haemorrhage remain the factors of concern.

Reynolds et al [84] *in* ***2003*** in their systematic review on comparing the variety of bone replacement grafts concluded that bone grafts increase bone level, reduce crestal bone loss, increase clinical attachment level and reduce probing depth compared to open flap debridement. Also, no differences in clinical outcome measures were observed between particulate bone allografts, and bovine derived xenografts.

2.1 Demineralized bone matrix (DBM)

It was first observed by ***Urist*** [105] in ***1965*** to induce heterotopic bone. The active components of DBM are a series of glycoproteins, transforming growth factor family (TGF-β) and bone morphogenic proteins (BMP). It demonstrates the property of osteoinduction and regulates morphogenic events involved in the development of tissue and organs. It stimulates the proliferation of undifferentiated mesenchymal cells through stimuli provided by demineralised bone matrix.

DBM has been used for several decades in human surgery in treatment of non-union, facial deformities, osteomyelitis and large defects resulting from tumor removal. ***Bingel***[8] *in* ***1999***, used DBM with good results for healing of fractures and bone defects in animal models.

Geesink et al[41] *in* ***1999***evaluated osteogenic activity of to demineralized bone matrix (grafton™) with controls in a human fibular defect. During the first postoperative year in the untreated group, no bony changes were observed while, in the Grafton DBM bone group, formation of new bone was visible from six weeks onwards.

Lee[60] ***in 2005***, compared the efficacy of different commercially available demineralised bone matrix substances in animal models. The results of this study suggest that DBM implants may enhance cementum regeneration in this defect model.

Wang[107] *in* ***2007*** **c**ompared the commercially available demineralized bone matrices for spinal fusion. There was no statistically significant difference between the rate of fusion after implantation of Osteofil™ and Grafton™.

Till date, the main delay in developing clinical products has been the need to find a suitable carrier to deliver the BMP to the site at which its action is required.

2.2 Bovine Derived Demineralized bone matrix

In recent times, a new demineralized bone matrix (DMBM; osseograft™) has been introduced. It is a sterile bioresorbable xenograft composed of type I collagen. It is prepared from bovine cortical bone samples, which result in nonimmunogenic flowable particles of approximately 250µm that are completely replaced by host bone in 4-24 weeks.[10]

Its xenogenic origin makes it an osteotropic matrix that provides improvement of bone formation by its chemical or structural characteristics in presence of osteogenic precursor cells. Xenogenic bone substitutes are osteoconductive in nature. They provide a scaffold to allow ingrowth and deposition of bone by osteoblasts from the margins of the defect on bone graft material**.**

The advantages of DMBM include being a totally resorbable space maintainer. It is deemed to be osteoconductive as well as oseoinductive in nature. It is cost effective and easy to handle and place.

A xenogenic graft is obtained from a donor of different species. Thus a limiting factor previously associated with the use of such materials was the potential of cross species antigenicity. However, the histological evaluation by ***Sogal, Tofe***[96] in ***1999*** confirmed the tolerance and good tissue acceptance of xenografts revealing no inflammation **a**nd almost completely free of risk of disease transmission .

Seyedin and Thomas[92] in ***1985*** isolated two naturally occurring peptides cartilage-inducing factors (CIF-A and CIF-B) from bovine demineralized bone by chemical treatment, that induce chondrogenesis.

Blumenthal et al[10] in ***1986*** studied the healing response of collagen gel in four dogs , evaluated it over 24 weeks and observed that collagen gel encouraged ingrowth of regenerative tissue-fibroblasts in the early stages of wound healing.

Choi[18] in ***1993*** reported that Type I collagen fulfils some of the criteria for bone formation. It stimulates osteoblast proliferation and differentiation of bone marrow cells. Since it is chemotactic for osteoblasts, fibroblasts and endothelial cells, it has been used for treatment of empty sockets, periodontal fenestrations and bone defects.

Garcia RR, Barbosa JR[39] in ***2000*** performed a histologic study of a bovine demineralized bone matrix on bone repair process in rabbits calvaria. Nine rabbits were used and two surgical defects were created on each calvaria, one was just filled with the animal blood and the other was filled with demineralized bone matrix. The animals were sacrificed at postoperative period of 3, 7, and 15 weeks. Specimens on light microscopy analysis revealed that bone repair was improved on cavities filled with bovine demineralized bone matrix.

Gupta, Pandit et al[45] in ***2007***, performed clinical and radiographic evaluation of an osseous xenograft for the treatment of intrabony defects. They assessed the effectiveness of DMBM at 40 sites on 30 patients at 3 months and 6 months post-operatively. In comparison to open flap debridement, significant improvements were observed in probing depths, clinical attachment levels and bone fill.

Kumaran et al[57] in ***2010***, compared the osteoblastic responses of commercially available demineralised bone matrices in an invitro study. They compared grafton™ and osseograft™ in bone marrow stem cell cultures (BMSCs) and observed an increased proliferative activity of osteoblasts in BMSCs in both cases for initial 5-10 days of culture. They also observed increased alkaline phosphatase activity when compared to control groups.

3. PLATELETS & PLATELET CONCENTRATES

Platelets are un-nucleated fragments of bone marrow megakaryocytes which circulate in blood for 8-10 days.[28] Historically, platelets are thought to contribute to the hemostatic process, where they adhere together to form a platelet plug in a severed vessel and actively extrude several initiators of the coagulation cascade.

Ross et al*[86]** in ***1974 introduced the regenerative potential of platelets by discussing their role in wound healing. The alpha granules of platelets contain various mitogenic factors such as platelet derived growth factor, vascular endothelial growth factor and transforming growth factor –β. This storage pool of growth factors proteins is vital to initial wound healing. Upon connective tissue contact, as occurs in injury or surgery, the cell membrane of the platelet is "activated" to release these alpha granules.

Active proteins are thus secreted which bind to transmembrane receptors of the target cells to activate intracellular signalling proteins. This results in expression of a gene sequence that directs cellular proliferation, collagen synthesis and osteoid production.[68]

3.1 *Platelet concentrates*

Application of fibrin adhesives in surgical management of haemostasis is well documented since early 1900s. These correspond to a natural biologic mechanism of fibrin polymerization, amplified in an artificial way. Thus yesteryear fibrin adhesives paved the way for the present day platelet concentrates.

Concentrating blood components via centrifugation provides with an opportunity to amplify the rich and advantageous components of patients own blood. Platelet rich plasma and platelet rich fibrin are two such emerging platelet

concentrates. These are basically fibrin matrices enmeshed with morphogenic proteins (growth factors) and leukocytes.

***Fabbro et al*[36]** summarised the ideal role of platelet concentrates as:

1. Augmentation of tissue healing : By increased proliferation of connective tissue progenitors that stimulate fibroblast and osteoblast activity and enhance osteogenesis.[67]
2. Anti-microbial activity :Against bacterial species involved in oral infections.[100]
3. Modification of host defence mechanism : By delivery of signalling peptides that attract macrophage cells.[65]
4. Modification of immune reaction: By releasing leukocytes that synthesize interleukins.[26]

3.2 *Platelet Rich Plasma*

The first generation of platelet concentrate, which consists of a limited volume of plasma enriched with platelets obtained from the patient, was called platelet rich plasma (PRP). If a normal human blood clot contains 5% platelets, according to **Sunitha *et al*** [99], a PRP blood clot contains 95% platelets.

PRP is known to contain growth factors such as PDGF and TGF – β, that may influence the regenerative process. Also in-vitro studies by ***Creeper et al*[24]** have reported proliferation of PDL and osteblastic cells under the influence of PRP.

Although PRP contains growth factors, their release in wound site tends to be rapid and for a short duration of time. Also, complex production protocol involving use of bovine thrombin and other biochemical agents has limited the benefits of platelet rich plasma.[88]

Lekovic et al[61] in ***2002***, for the management of intrabony defects, compared platelet rich plasma, bovine porous bone mineral and guided tissue regeneration with a combination of platelet rich plasma and bovine porous bone mineral for the management of intrabony defects. They reported effective results in both groups. However, GTR appeared to add no clinical benefit to PRP and BPBM.

Hanna R et al[47] in ***2004*** compared the clinical outcomes obtained by the combination of PRP and a bovine derived xenograft (BDX) to those obtained from the use of the bone replacement graft alone, in a 9 months clinical trial. The addition of a high concentration of autologous platelets to a bovine derived xenograft to treat intrabony defects significantly improved their clinical periodontal response.

Dori et al[33] in ***2007*** studied the effect of platelet rich plasma on the healing of intrabony defects treated with a natural bone mineral and collagen membrane. The result concluded equally significant clinical outcomes in both the groups. However, the use of PRP failed to improve the results obtained with combination of PRP and natural bone mineral.

Dori et al[34] in ***2007*** evaluated the effect of platelet rich plasma on the healing of intrabony defects treated with an anorganic bovine bone mineral and expanded polytretrafluoroethylene membranes. Optimal clinical results were obtained with ABBM+GTR with or without the addition of PRP.

Piemontese[77] in ***2008*** treated periodontal intrabony defects with demineralized Freeze-Dried Bone Allograft in combination with Platelet-Rich Plasma. Treatment with a combination therapy led to a significantly greater clinical improvement in intrabony periodontal defects compared to DFDBA with saline.

However, no statistically significant differences were observed in the hard tissue response between the two treatment groups, which confirmed that PRP had no effect on hard tissue fill or gain in new hard tissue formation.

Parimala et al[75] in ***2010*** performed a comparative evaluation of bovine porous bone mineral with and without platelet rich plasma. Significant results were observed with both treatment modalities in probing depth reduction, gain in clinical attachment level and amount of defect fill. Although the mean difference between two groups was statistically non-significant, more favourable results were observed with combination therapy.

The potential benefits of PRP have been variable in literature. Although some authors reported significant improvements in tissue healing and bone formation using PRP[67, 6, 86]**,** others failed to observe improvement.[83,46] Benefits of treating intrabony periodontal defects with PRP combined with bone mineral were reported. However, the final consensus of PRP on bone grafts remains questionable.

Thus, the technical and regenerative limitations of platelet rich plasma led to the discovery of a better, completely autologous fibrin matrix called Platelet Rich Fibrin**.**

3.3 *Platelet Rich Fibrin*

A second generation platelet concentrate, developed in France in 2001 by ***Choukroun.et al*** [19], is an autologous growth factor reservoir which attempts to accumulate platelets and cytokines in a physiologic fibrin clot.

PRF clot concentrates 97 % of platelets and >50 % of leukocytes in a specific three dimensional distribution. It consists of intimate assembly of cytokines, glycanic

chains and structural glycoproteins enmeshed within a slowly polymerized fibrin network.[28]

3.3.1 Significance of PRF :

1. **Role of fibrin matrix**

A soluble fibrillary molecule, fibrin is an activated form of plasmatic molecule fibrinogen that is massively present both in plasma and in the platelet alpha granules which is transformed into an insoluble fibrin by thrombin. The polymerized fibrin gel constitutes the first cicatricial matrix of the injured site.[27, 28]

The three dimensional structure of the matrix resembles that of physiologic fibrin.[31] The enmeshed cytokines influence the extracellular matrix which allows migration, division and phenotypic change of endothelial cells, thus leading to angiogenesis.[50]

2. **Role of platelets and growth factors**

Periodontal regeneration is a multi-factorial process and requires an orchestrated sequence of biological events including cell adhesion, migration, multiplication and differentiation.[43]

The scientific rationale behind the use of platelet concentrates lies in the fact that the platelet α granules are a reservoir of many growth factors (GFs) that are known to play a crucial role in hard and soft tissue repair mechanism[3,13]**.** Platelet growth factors exhibit chemotactic and mitogenic properties that promote and modulate cellular functions involved in tissue healing, cell proliferation and regeneration **.**[5]

The growth factors released by α granules encompass a group of cytokine polypeptides with relatively low molecular weight ranging from 6-45kDa. PRF growth factors include Platelet derived growth factors (PDGFs), Transforming growth

factor –β (TGF-β), Vascular endothelial growth factor (VEGF), Epidermal growth factor (EGF) and Insulin-like growth factor -1(IGF-1).[28]

a. **PDGF : I**t was first described by ***Ross et al*** [86] in ***1974*** as a factor which accelerate proliferation of monkey arterial smooth muscle cell in cell culture. The types of PDGF include PDGF AA, BB, AB, CC, DD the classic dimmers being AA, AB and BB.

   PDGF AB and BB are released from platelets at injury site whereas AA isoform is secreted by unstimulated osteoblastic cell lineages.[14]

   PDGF plays an essential role in regulation, migration, proliferation and survival of mesenchymal cell lineages. It has mitogenic effects on stem cells and osteoblasts, stimulates pre-mitotic partially differentiated **osteoprogenitor cells**, stimulates cell replication of endothelial cells and promotes angiogenesis. It modulates the effects of other growth factors and promotes perivascular healing of the wound. It also plays a crucial role in mechanisms of physiologic cicatrisation. [17, 51]

b. **TGF –β :** Of the three isoforms TGF-β1 is the most significant. It is an inflammatory regulator and the most powerful fibrosis agent amongst all cytokines.[12]

   TGF-β1 and TGF-β2 activate fibroblasts, which undergo cell division and produce collagen [66]; control cellular differentiation and proliferation of cementoblasts; activate osteoprogenitor cells and further differentiates them to produce bone matrix; activate endothelial cells to produce new capillaries; stimulate mesenchymal stem cells to induce mitosis so as to provide the large population of wound healing cells needed for completion of healing.

c. **VEGF : I**t is the most powerful and omnipresent known vascular growth factor. The main role is in initiation of angiogenesis

d. **IGF -1 :** Although present mainly in plasma it exerts chemotactic effects towards human osteoblasts[64], regulates cell migration, proliferation , differentiation and matrix synthesis. Acts as cell multiplication mediators in apoptosis by inducing survival signals protecting cells.

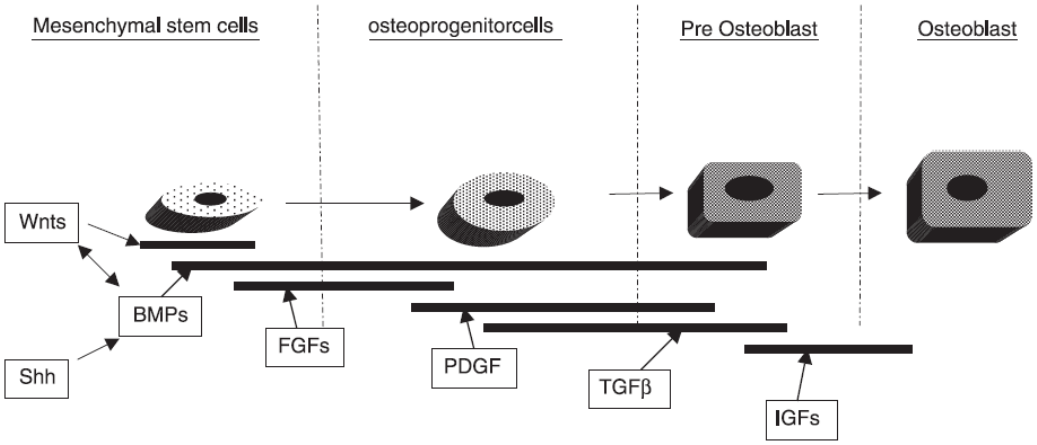


Figure 1 : Stages of osteoblast lineage with different growth factors
(*Hughes et al*)[51]

3. Role of leukocytes

Fibrin mesh provides natural immunity under the influence of fibrinogen degradation products (FDP) that stimulate the migration of neutrophils, modulates phagocytosis and enzymatic degradation of the neutrophils. Also chemotactic agents trapped in fibrin control wound colonization by macrophages.[26]

Leukocytes trapped in PRF have anti-infectious effect and act as an immune regulation node. PRF contains all key immune cytokines like IL 1β, IL 6, IL 4 and TNF. [26] They have the ability to control the inflammatory response at the wound site.

Kawamura et al[56] also demonstrated that PRF may act as supportive matrix for BMPs. Thus, indicating role of PRF on wound healing.

3.3.2 Parts of PRF

As suggested by ***Choukroun***[19], centrifugation of patients blood results in into three separate layers i.e blood clot at the bottom, fibrin matrix in the middle (PRF) and platelet poor plasma on top.

Histological analyses by ***Dohan et al***[28] *in* ***2006*** determined the platelet distribution within the various layers of the centrifuged blood: The platelets accumulate in the lower part of the fibrin clot, mainly at the junction between the red cells (red thrombus) and the PRF clot itself. This observation highlights the finding that the red extremity of PRF would be more effective than the higher part of the fibrin clot and would be of maximum clinical application.

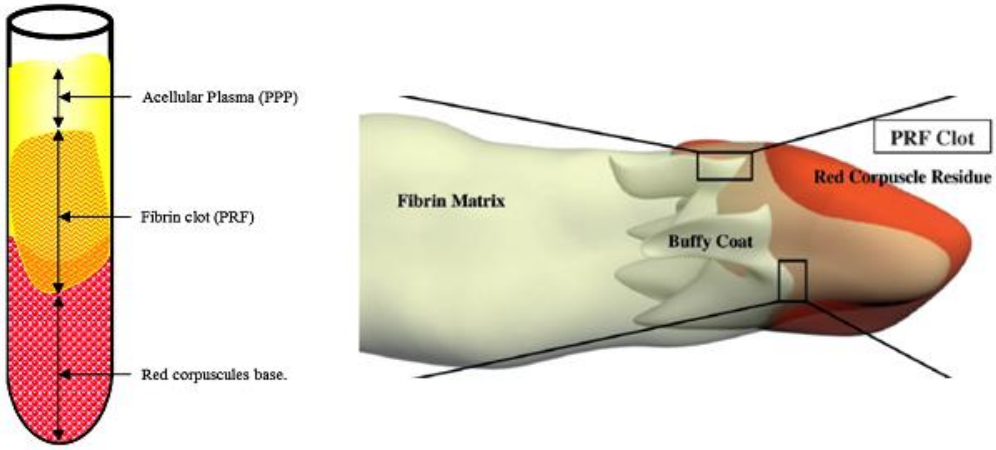


Figure 2 : Parts of Platelet Rich Fibrin[28]

3.3.3 Activation

Activation and degranulation of platelets is important to initiate and support their aggregation at the healing site. Given the absence of anticoagulant, activation of platelets in contact with silica of glass tube walls starts the coagulation cascade.

Fibrinogen forms fibrin in the presence of physiologic thrombin. Post centrifugation fibrin is obtained in the middle of the tube with massively concentrated platelets

Activation of platelets, thus releases the cytokines (IL-1 beta, IL-6, TNF-alpha) and growth factors (TGF beta 1, PDGF, VEGF, EGF) that stimulates cell migration and proliferation within the fibrin matrix and thus begins the first stage of healing.[26]

3.3.4 Technical significance

According to a study by ***Su et al***[98] in ***2009***, platelet rich fibrin allows continuous release of growth factors for over 300 minutes following its preparation. Hence, it must be used immediately after preparing. The progressive release of cytokines and leukocytes continues for a period of 7-11 days, as the fibrin network disintegrates.[95]

Slow and natural polymerization of PRF in the presence of physiologic thrombin gives it the crucial three dimensional organization of fibrin network. This characteristic fibrin network provides it with great elasticity, thus forming a very strong PRF membrane. Waiting for more than a minute or two may cause the fibrin to polymerize in a diffuse way, leaving behind only a small poorly formed clot in the test tube.[27]

3.3.5 The various **advantages** of PRF include **(*Dohan et al*)** [26,27,28,30]:

1. Completely autogenous
2. Extended growth factor release for 7 days
3. Simple and faster technique
4. In-expensive
5. No requirement of any additive constituent such as bovine thrombin

6. No biochemical handling involved
7. No associated immune reactions
8. No associated infections
9. Acts as an 'immune regulation node'
10. Has anti- inflammatory effects

3.3.6 Limitations

Connell[23] in ***2007*** raised concern regarding the safety issue of PRF methodology. He commented on the types of tubes to be used to produce PRF and the possible hazards of silica containing glass tubes.

However, **Dohan *et al***[29] in the same year conducted a cytotoxicity analysis of PRF on wide range of human cells and concluded that silica microparticles coating these tubes are not cytotoxic for the tested human cells. They also reported improved mitotic proliferation and suggested contact with silica is necessary to start the polymerisation process as silica behaves as clot activator. Thus, to produce PRF either dried glass tubes or glass coated plastic tubes must be used.

Other sensitive issues not yet revealed, that may influence the nature of PRF include variation in quantity and quality of PRF with aging, influence of systemic diseases (thrombocytopenia, bleeding disorders, diabetes, leukocyte adhesion syndromes etc), nutrition, environmental or racial differences, blood profile, autoimmunity and genetic predisposition.

PRP v/s PRF

According to ***Mosesson et al***[73], who described the structural and biological features of fibrinogen and fibrin in detail, the 3-dimensional organization of fibrin network depends on activation mechanism.

1. Strong concentration of thrombin leads to condensed tetramolecular or bilateral junctions in turn causing thickening of fibrin polymer. This rigid network is not very favourable for cytokine enmeshment and cellular migration. However, it can seal biologic tissues well. Such a structural organization is observed in platelet rich plasma (PRP).

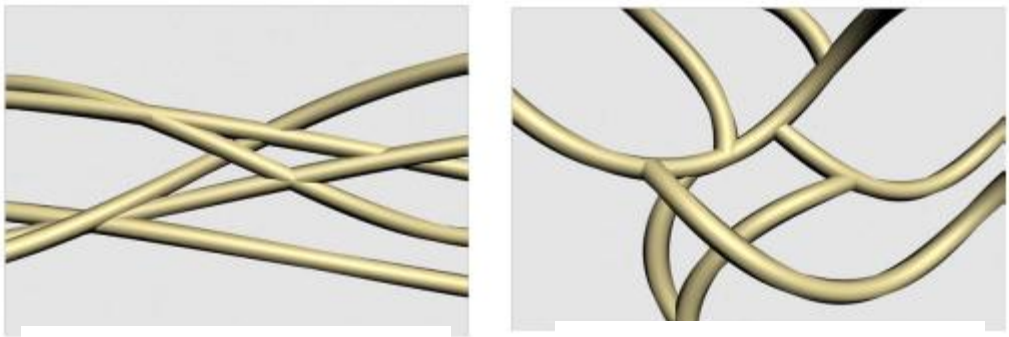


Figure 3 : Structural Organization of PRP and PRF

2. Weak concentration of thrombin leads to trimolecular or equilateral junctions which result in a fibrin matrix that is flexible and can support cytokine and cellular migration. Such a flexible elastic and very strong network is seen in platelet rich fibrin (PRF).[31] Unlike PRP, PRF results from a natural and progressive polymerization that occurs during the centrifugation process.

In an in- vitro comparison of PRF with PRP, **He *et al*[48]** *in* **2009** demonstrated gradual extended release of autologous growth factors and better induction of osteoblastic differentiation and proliferation by PRF.

In a clinical trial by ***Pradeep et al***[79] in ***2012*** comparative evaluation of autologous PRF and PRP in intrabony defects demonstrated equally favourable clinical and radiographic results in both groups when compared to open flap debridement alone.

But given the inexpensive nature, less time consuming and less technique sensitive and favourable properties of PRF matrix, it is considered as a better choice among the two available varieties of platelet concentrates.

Applications:

The vast benefits of PRF have led to its applications in different fields of medicine and dentistry:

1. Ear, nose, throat and plastic surgery[91]
2. Oral and maxillofacial surgery[20,21]
3. Pre-implant and implant surgery [69]

Mazor Z et al[69] in ***2009,*** used PRF as the sole agent in simultaneous sinus lift and implant placement. They demonstrated stabilization of high volume of natural bone in the sub-sinus cavity.

Toffler et al[102] in ***2009*** advocated membrane insurance by possibly sealing an undetected perforation during lateral window osteotomy procedure using PRF membrane

Simonpieri et al[95] in ***2009*** reported maxillary reconstruction using FDBA, PRF membranes and 0.5% metronidazole solution. Metronidazole solution provided a proficient protection of the bone graft against unavoidable bacterial contamination.

Chang et al[16] in ***2010*** reported in their in-vitro study that platelet rich fibrin can modulate the expression of extracellular signal-regulated protein kinase and osteprotegrin in human osteoblasts, suggesting potential role in bone regeneration

Kang et al[55] in ***2011*** strongly supported the characteristics of PRF as a bioscaffold and reservoir of growth factors for tissue regeneration.

Periodontal Applications :

1. As a resorbable membrane for recession coverage[7, 54]
2. As a scaffold for human periosteal tissue and bone tissue engineering[40]
3. As a sole grafting material in osseous defects[81]
4. As a grafting material in combination with other grafts in osseous defects[62,78]

***Kankamendela et al*[54]** in **2009** reported platelet rich fibrin as a potential novel root coverage approach for covering localised gingival recession in mandibular anterior teeth using combined laterally positioned flap technique and PRF membrane.

***Gassling et al*[40]** in **2010** in their in-vitro study compared of PRF with the commonly used collagen membrane Bio-Gide® as scaffolds for periosteal tissue engineering**.** The proliferation level as measured by quantitative and qualitative revealed higher values for PRF. Thus, suggesting superior nature of PRF to collagen (Bio-Gide®) as a scaffold for human periosteal cell proliferation and bone tissue engineering.

Pradeep et al*[81]** in ***2011 in their clinical trial compared autologous platelet rich fibrin to open flap debridement alone in treatment of 3-Wall Intrabony Defects in Chronic Periodontitis patients. They observed mean reduction in probing depth greater in test group (4.55 ± 1.87 mm) than control group (3.21 ± 1.64 mm) while mean PAL gain was also found to be greater in test group (3.31 ± 1.76 mm) compared to controls (2.77 ± 1.44 mm). Furthermore, significantly greater percentage of mean bone fill was found in the test group (48.26 ± 5.72 %) compared to control (1.80 ± 1.56 %).

Thorat et al*[101]** in ***2011 performed a controlled clinical trial in order to estimate the clinical effects of autologous PRF in treatment of intra-bony defects**.** All the

clinical parameters and radiographic parameters reported greater improvement with the use of PRF. Significant reductions in probing depth, CAL gain and greater intrabony defect fill was observed.

4. COMBINATION APPROACHES

Choukroun's PRF has revolutionised the field of regenerative dentistry and motivated the researchers and clinicians further to apply this procedure along with tissue engineering protocol

PRF membranes protect the surgical site and promote soft tissue healing and PRF fragments when mixed with graft material may function as a "biological connector" between the different graft elements, and as a matrix that supports neo-angiogenesis, capture of stem cells and migration of osteoprogenitor cells to the centre of the graft.[102]

Although the additional benefits of PRF seem to be revolutionizing, only limited research is presently available on PRF in combination with bone grafts in comparison with bone grafts alone

In a **2005** study **S*ammartino***[87]**,** reported the use of autogenous bone mixed with platelet-enriched fibrin glue for simultaneous implant placement in dogs. The combination group demonstrated enhanced osseointegration and better bone formation than use of autogenous bone alone.

Choukroun et al[21] in ***2006*** attempted to evaluate the potential of PRF in combination with freeze-dried bone allograft (FDBA) to enhance bone regeneration in sinus floor elevation. After 4 months of healing time, histological maturation of the test group appeared to be identical to that of the control group which was for a period

of 8 months. Moreover, the quantities of newly formed bone were equivalent between the 2 protocols**.**

Meyer et al*[71]** in ***2009 advocated that the long-term reliability of β TCP associated to growth factors (PRP or PRF) without bone graft, in massive sinus-lift procedures induces fewer complications, and the implant success as well as resorption rate is comparable to the one obtained by using autologous bone grafts.

Kanakamadela et al*[53]** in ***2009 reported combined use of platelet rich fibrin and bone graft has for combined periodontic – endodontic furcation defect. They reported beneficial results with the combination therapy.

Simonpieri et al*[95]** in ***2009 summarised the use of platelet concentrate PRF with bone grafting can offer various advantages :

1. Mechanical protection of grafted materials with PRF membrane.[93]
2. PRF fragments act as biological connectors between graft materials.[102]
3. Fibrin network facilitates cellular migration, neoangiogenesis, vascularisation and survival of graft [20,28]**.**
4. Platelet cytokines are gradually released as fibrin matrix is resorbed[69]
5. Self regulation of inflammatory phenomenon within the grafted site given the presence of leukocytes and cytokines.[37]

Jang et al*[52]** in ***2010 determined the ability of silk fibroin powder as biomaterial template for the regeneration of peri-implant defects when mixed with ***Choukroun's*** PRF in ten New Zealand white rabbits. Histomorphometric analysis show greater bone formation and repair of per-implant defects for experimental group (silk fibroin and PRF) than in control (unfilled) group.

Lekovic et al*[62]** in ***2012 compared PRF and bovine porous bone mineral vs PRF alone in the treatment of intrabony periodontal defects. The results of this study indicated that PRF can improve clinical parameters associated with human intrabony periodontal defects. They also observed that the combination of BPBM with PRF had the ability to augment the effects of PRF in reducing pocket depth, improving clinical attachment levels and promoting defect fill.

Pradeep et al*[78]** in ***2012 combined porous hydroxyapatite graft with platelet rich fibrin in management of intabony defects in chronic periodontitis patients. The study aimed to explore the additional effectiveness of autologous PRF with bone graft material. On evaluation HA addition to PRF increased the regenerative effects than observed with PRF alone.

Thus, given the various advantages of platelet rich fibrin as a replacement material and its possible additional benefits in management of periodontal osseous defects, the present study was undertaken. It involved the comparative assessment of the efficacy of DMBM (osseograft^{TM}) alone and in combination with platelet rich fibrin.

MATERIALS AND METHODS

The study population was selected from the Outpatient Section of the Department of Periodontics , Tamil Nadu Government Dental College and Hospital, Chennai, India.

Inclusion criteria

- Patients willing for voluntary participation & have signed informed consent.
- Patients with age group 20-45 years of either gender
- Systemically healthy subjects
- Patients with pocket probing depth ≥5mm following phase I therapy
- Radiographic evidence of vertical bone loss.

Exclusion criteria

- Patients showing unacceptable oral hygiene maintenance during pre-surgical (phase-I) period
- Patients with history of periodontal therapy - 6 months prior to study
- Patients under any medication - 6 months prior to study
- Patients with use of tobacco or tobacco related products
- Pregnant / Lactating patients
- Patients with known systemic diseases
- Patients with any known metabolic disorders
- Patients with any known allergies

STUDY DESIGN

Ethical clearances were obtained from the institution's ethical committee and the ethical principles were meticulously followed throughout the course of the study.

Subjects for the study were selected randomly, with no discrimination on the basis of sex, caste, religion or socioeconomic status. After explaining the study procedure (*Annexure 1*), written informed consent was obtained from all the subjects selected for the study (*Annexure 2 & 3*). Examination was preceded by a thorough medical and dental history of the subjects. Each subject underwent full-mouth periodontal probing and charting, and radiographic evaluation (*Annexure 4*).

A total of 18 sites in 15 subjects were randomly selected and divided into two groups :

Group I : 9 sites treated with bone graft (DMBM) alone

Group II : 9 sites treated with combination of bone graft (DMBM) and platelet rich fibrin.

STUDY PROTOCOL

1. Institutional ethical committee approval.
2. Medical history and informed consent.
3.Complete periodontal examination using a mouth mirror and a Williams periodontal probe under artificial light.
4. Intra-oral evaluation and periodontal examination using clinical parameters namely Gingival bleeding index, Plaque index, Pocket probing depth and Clinical attachment level
5. Radiographic evaluation
6. Phase I therapy and re-evaluation of clinical parameters after 4-6 weeks

7. Selection of study sites and random allocation into two groups
8. Surgical procedures (open flap debridement and graft placement) according to the group selection
9. Post –operative care
10. Clinical re-evaluation at the end 6 months
11. Radiographic re-evaluations at the end of 3 and 6 months

PRE-OPERATIVE CLINICAL ASSESSMENT

The clinical parameters evaluated before and after phase I therapy and 6 months post surgically included :

1. Plaque index
2. Gingival bleeding index
3. Probing pocket depth in mm (PPD)
4. Clinical attachment level in mm (CAL)

Plaque Index *(Silness and Loe 1964***)** [93]

All teeth were examined at 4 sites each (disto-facial, facial, mesio-facial lingual / palatal) and were scored as follows :

Criteria for Scoring:

Score 0 No plaque

Score 1 Plaque not visible to the naked eye, detected only by running the explorer or by using a disclosing agent

Score 2 Thin to moderate accumulation of soft deposits within the gingival pocket or on tooth and gingival margin, visible to the naked eye

Score 3 Abundance of soft matter within gingival pocket and / or on tooth surface and margin, inter-dental area stuffed with soft debris

Calculation : Plaque index per tooth = Total score / 4

$$\text{Plaque index per individual} = \frac{\text{Total P I per tooth}}{\text{Total number of teeth examined}}$$

Interpretation: Score 0 – Excellent oral hygiene

0.1 to 0.9 – Good oral hygiene

1.0 to 1.9 – Fair oral hygiene

2.0 to 3.0 - Poor oral hygiene

Gingival Bleeding Index* (Ainamo & Bay 1975***)** [1]

Starting distobuccally, the probe was inserted slightly into the sulcus and run to the buccal and mesial surfaces of every tooth at an angle of about 45^{o}. This was repeated for all teeth present. Probing was similarly carried out at palatal/lingual sites. Any gingival units that exhibited bleeding were recorded. The total number of bleeding sites per tooth was thus recorded for every tooth except the third molar.

Criteria for Scoring

Positive score (1) - Presence of bleeding within 10 seconds

Negative score (0) - Absence of bleeding

$$\% \text{ of bleeding sites} = \frac{\text{Total number of positive score}}{\text{Total number of surfaces of all teeth}} \times 100$$

Stent Preparation

Acrylic occlusal stents were fabricated over the study models. Self cured pink acrylic was used for the purpose. The stent covered the occlusal and coronal 1/ 3rd of the labial and lingual surfaces of the teeth. It involved one tooth mesially and one distally to the study tooth. Vertical grooves were made to guide the placement of the probe in the same plane and direction repeatedly during measurements to avoid any variation. The recordings were made using a Williams periodontal probe.

Probing Pocket Depth (PPD)(In mm) [15]

Probing Pocket Depth was measured from the gingival margin to the base of the pocket in millimeters using Williams Periodontal Probe. The probe was walked within the gingival sulcus along the circumference of the tooth. Keeping the probe parallel to the long axis of the selected tooth, six measurements were made per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, and Distolingual).

Clinical Attachment Level (CAL) [15]

Clinical Attachment Level was measured from the Cemento – Enamel Junction (CEJ) to the base of the pocket using Williams Periodontal Probe. The probe was placed parallel to the long axis of the tooth and readings were recorded at six different sites (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, and Distolingual).

PRE-SURGICAL EVALUATION

Patients to be included in the study were selected according to the above defined criteria. All the selected patients were given oral hygiene instructions and were subjected to phase-I periodontal therapy. After 3-4 weeks of phase-I therapy re-evaluation of the clinical status was performed and patients with acceptable oral hygiene (PI ≤ 1) were selected. After correlating with radiographic findings surgical procedure was planned. The infrabony defects were randomly assigned to either group I or group II.

***SURGICAL PROCEDURE*:**

Intra-oral antisepsis and extraoral antisepsis was performed with 0.2% chlorhexidine digluconate rinse and 5% povidone iodine solution respectively. The operative site was anaesthetized with 2% Lignocaine HCl with adrenaline (1:80,000) using block and infiltration techniques.

Crevicular incisions were made on the facial and lingual/palatal surfaces, extending on tooth on each side of the defect tooth using the Bard Parker blade No.15. A full thickness mucoperiosteal flap was reflected using the periosteal elevator. Care was taken to preserve maximum amount of interdental papillary tissue. After reflection of the flap and exposure of osseous defect, a thorough surgical debridement of soft and hard tissue was done using the area specific Gracey curette. No osseous recontouring was performed. Debridement was followed by copious 0.9% normal saline irrigation.

In group I, the defect was filled with DMBM (osseograft^{TM}) mixed with saline. In group II, the defect was filled with a combination of PRF and DMBM (osseograft^{TM}). Freshly prepared PRF gel was obtained after centrifugation and immediately used. Following light squeezing between two sterile gauze pieces, it was

made into small pieces and mixed with equal proportion of bone graft (1:1 v/v).[62] The mixture was then placed into the osseous defect with light pressure till it filled upto the most coronal level of osseous wall.

The mucoperiosteal flaps were repositioned and secured using 3-0 black silk braided sutures. Periodontal dressing (Coe-pac™) was placed. All patients were prescribed systemic antibiotics (amoxicillin 500mg thrice daily, metronidazole, 400mg twice daily) and analgesics (paracetamol 500 mg thrice daily).

Post operative instructions were given to all the patients. Re-evaluation for any acute signs of inflammation or infection was done at 24 hours post surgically. 7 days following surgery, the dressing and sutures were removed and surgical site was irrigated with normal saline. Patients were observed for any signs or symptoms of post operative complications. Patients were reviewed every week for the first four weeks. Thereafter depending on patients' maintenance, recall appointments were made after 3 months and finally at 6 months and radiographs were repeated. During the entire follow-up period, oral hygiene maintenance was reinforced and supragingival scaling was performed, if required.

PRF preparation [19, 27]

Platelet rich fibrin (PRF) was prepared in accordance with the protocol developed by ***Choukroun et al***. Just prior to surgery approximately 5-6ml of intravenous blood was drawn from the cubital fossa of the patient. Whole blood was collected in a 10-ml sterile glass tube without anticoagulant and immediately centrifuged at 3000 rpm for 10 minutes.

Blood centrifugation resulted in separation of blood into a structured fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma (Platelet-poor plasma) at the top.[27] After removal of PPP, PRF was easily separated from red corpuscles base [preserving a small red blood cell (RBC) layer] using sterile tweezers and scissors.

***RADIOGRAPHIC MEASUREMENTS*:**

Intraoral periapical radiographs were taken for each site using long cone paralleling technique and XCP holders at baseline, 3 months and 6 months post-operatively and evaluated.

The radiographs were digitized using digital camera[82] (canon powershot sx230 HS), and images were analysed using corelDRAW software version x6[89].

The following anatomical landmarks (Photograph 10a) of the intrabony defect were identified on the radiograph images based on criteria set by ***Bjorn et al*** [9] and ***by Schei et al*** [90] :

1. CEJ: The cemento-enamel junction of the tooth with the intrabony defect.
2. AC: The most coronal position of the alveolar bone crest of the intrabony defect when it touches the root surface of the adjacent tooth before treatment, the top of the crest.
3. BD : The most apical extension of the intrabony destruction where the periodontal ligament space still retained its normal width before treatment, the bottom of the defect.

If restorations were present, the apical margin of the restoration was used to replace the CEJ as a fixed reference point.

For measurements, connector line tool of the software was used. A line was drawn from CEJ to base of the defect and a perpendicular was then drawn from alveolar crest to this line to obtain the distance between CEJ and alveolar crest. Also, a line was drawn from CEJ to root apex (Photograph 10b). All measurements were recorded in millimetres.

The following linear measurements were performed [63,82] :

1. CEJ to bottom of the defect (CEJ to BD) = Defect Depth (DD)
2. CEJ to most coronal extent of the inter-dental alveolar crest (CEJ to AC)
3. Depth of the intrabony defect at baseline = (CEJ to BD) - (CEJ to AC)
4. Correction factor : In order to estimate distortion between the consequent radiographs, an anatomically non-variable distance i.e. the root length (distance from the CEJ to the root apex (CEJ to RA)) was measured on all the radiographs. The correction factor (CF) was calculated as follows:

   $$\frac{\text{CEJ to RA (baseline)}}{\text{CEJ to RA (post-op)}} = \text{Correction Factor}$$

   In case it was not possible to measure the root length, the crown length was assessed (distance from the incisal margin of the crown to the CEJ).

5. Bone fill (BF) = CEJ to BD (baseline) --- [CEJ to BD (post op) x CF]
6. Bone fill percentage (BF %) = $\frac{\text{Bone fill}}{\text{Defect Depth (at baseline)}} \times 100$
7. Bone crest change(BCC) = CEJ to AC (baseline) - [CEJ to AC (post op) x CF]
8. Bone crest change percentage (BCC %) = $\frac{\text{Bone Crest change}}{\text{CEJ – AC (baseline)}} \times 100$

If the results were negative, this meant that a process of bone resorption had occurred.[63]

9. Amount of original defect resolution (DR) = Bone fill (BF) – bone crest change (BCC)

10. Percentage(%) of original defect resolution = $\frac{\text{Defect Resolution}}{\text{Depth of intrabony defect (Baseline)}} \times 100$

All the above made observations were recorded and subjected to statistical analysis.

ARMAMENTARIUM

For clinical examination

- Mouth mirror
- Williams periodontal probe
- Curved explorer
- Dental tweezers
- Kidney tray
- Cotton roll
- Sterilized disposable gloves
- Disposable facemask

For Phase I Therapy

- Mouth Mirror
- Explorer
- Scalers and Curettes
- Kidney Tray
- Cotton Rolls
- Disposable Gloves, facemask and headcap
- Disposable syringe
- Local Anaesthetic solution
- Aspirating Needle

For PRF preparation and collection **:**

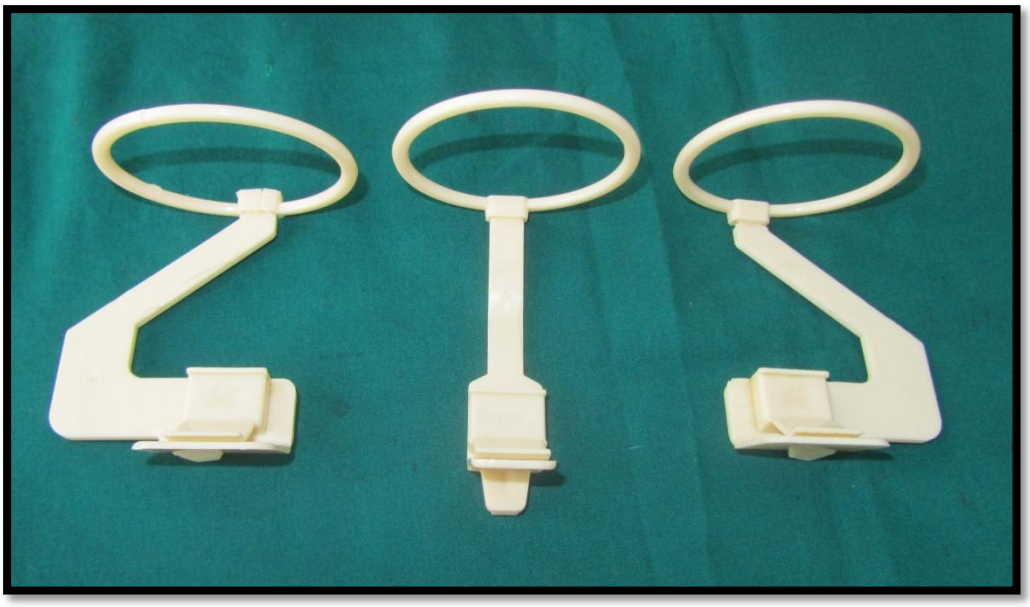
- Sterile cotton and surgical spirit.
- Disposable syringe
- Tourniquet
- Sterile glass test tube
- Centrifuge
- Dental tweezers and scissors

For surgical procedure :

- Mouth mirror
- Williams periodontal probe
- Dental tweezers
- Surgical gloves
- Disposable mouth mask
- Local anaesthesia
- Bard parker blade no 15 and handle – straight and contra-angled.
- Periosteal elevator
- Area specific Gracey's curettes and universal curette (Columbia 4R-4L)
- Straight and Curved scissors
- Saline and irrigation syringe
- Dapen dish
- DMBM (osseograft ™)
- Suture material – 3-0 black silk braided
- Needle holder
- Cement spatula and Glass slab
- Periodontal dressing (Coe pac™)

Photograph 1 :Surgical Armamentarium

Photograph 2 : Demineralized Bone Matrix (DMBM)



Photograph 3 : XCP Holders

Photograph 4 : Centrifuge

GROUP I – OPERATIVE VIEWS

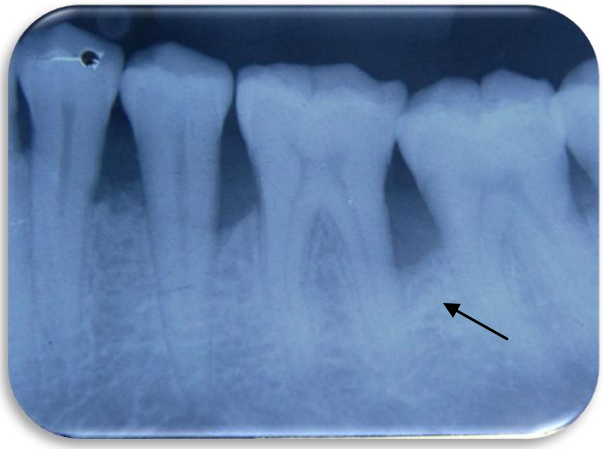
Photograph 5 (a) : Pre-operative view

Photograph 5(b) : Intra-operative view

Photograph 5(c) : After placement of DMBM

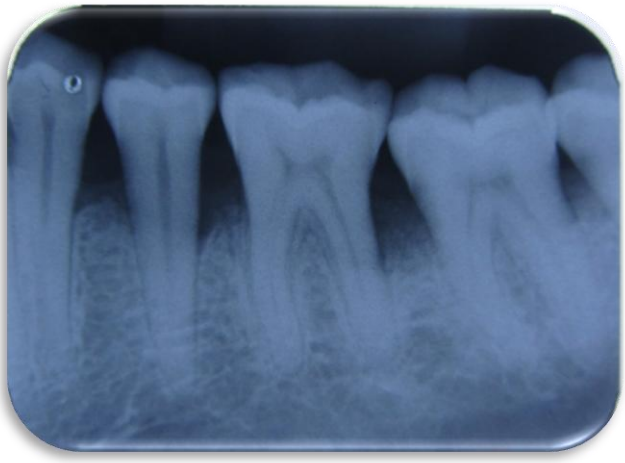
Photograph 5(d) : Post Operative view

GROUP I – RADIOGRAPHIC VIEWS



Photograph 6(a) : At Baseline

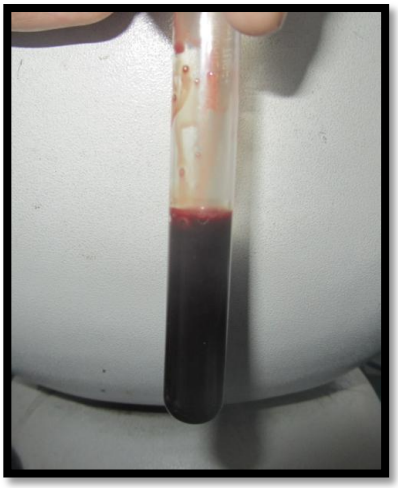
Photograph 6(b) : At 3 months



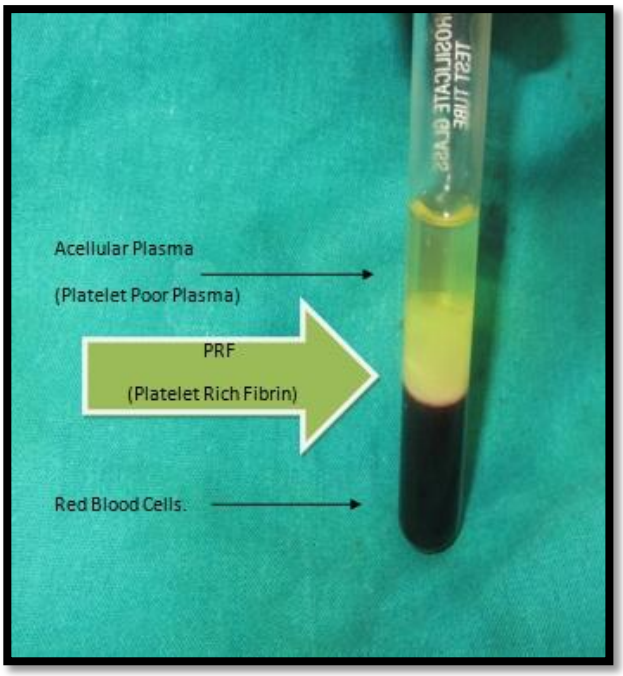
Photograph 6(c) : At 6 months

ARMAMENTARIUM FOR PRF COLLECTION AND MIXING WITH DMBM

Photograph 7(a) : Blood Collection Kit



Photograph 7(b) : Blood immediately before centrifugation



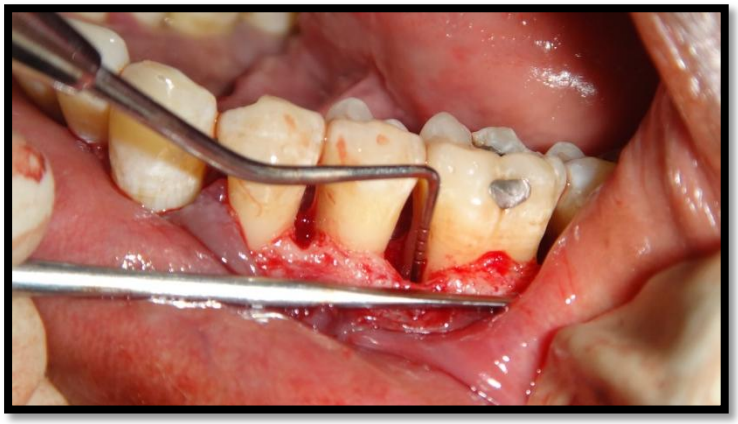
Photograph 7(c) Immediately after centrifugation

Photograph 7(d) PRF

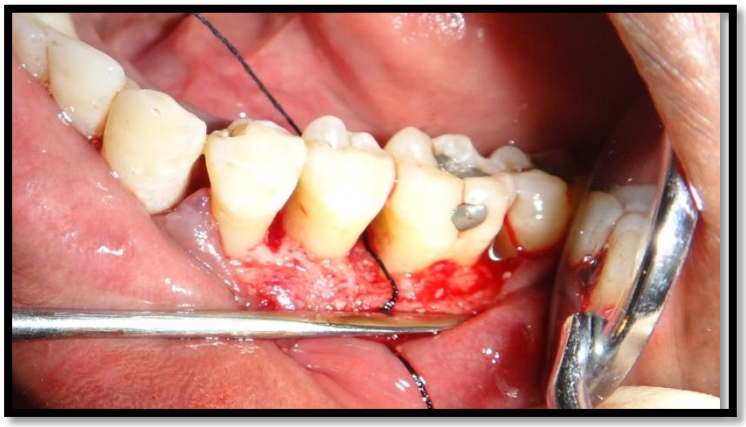
Photograph 7(e) PRF+DMBM

GROUP II – OPERATIVE VIEWS

Photograph 8(a) : Pre-operative view



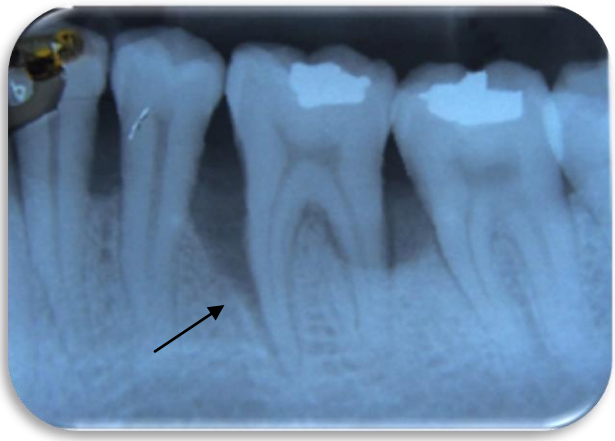
Photograph 8(b) : Intra-operative view



Photograph 8(c) : After placement of PRF+ DMBM

Photograph 8(d) : Post Operative view

GROUP II : RADIOGRAPHIC VIEWS



Photograph 9(a) : At Baseline

Photograph 9(b) : At 3 months

Photograph 9(c) : At 6 months

RADIOGRAPHIC MEASUREMENTS

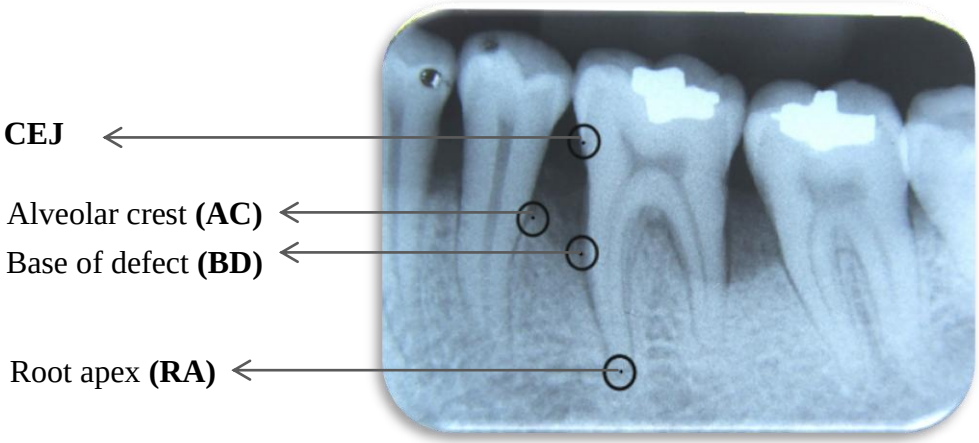


Figure 10(a) : Marking the landmarks

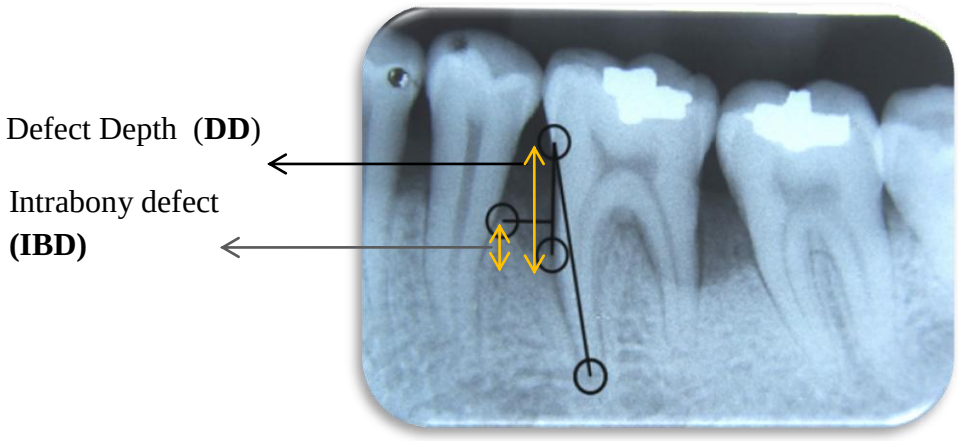


Figure 10(b) : Measurement of radiographic parameters

STATISTICAL ANALYSIS

The statistical analysis was done using the computer software program SPSS version 16.0 (Statistical Package for Social Science, Version 16). Descriptive data are presented as mean ± SD and range values.

The comparison of mean values was done using Wilcoxon Signed ranks test for within a group (intra-group analysis) and Mann-Whitney U test for intergroup comparisons to calculate the p-value. For the multivariate analysis Kruskal-Wallis test was used to find the significance between the groups.

In all the above statistical tools the probability value $P \leq 0.5$ was considered as significant level.

STATISTICAL FORMULA'S USED FOR DATA ANALYSIS.

Wilcoxon Signed Rank Test

The formula is

$$Z = \left[T - \frac{n(n+1)}{4}\right] / \sqrt{\left[\frac{n(n+1)(2n+1)}{24}\right]}$$

where n is the number of pairs. T is the sum of the ranks for the positive differences. We compare Z to the tabulated Normal distribution.

Mann-Whitney U-Test

The formula is

$$Z = \left[T - \frac{n1(n1+n2+1)}{2}\right] / \sqrt{n1n2(n1+n2+1)/12}$$

where n1 and n2 are the sample sizes in Group I and Group II respectively. T is the sum of the ranks for the n1 observations.

Kruskal-Wallis Test:

Kruskal-Wallis one way analysis of variance is applied to populations from which the samples drawn are not normally distributed with equal variances.

The test statistic is computed from the following formula:

$$H = \frac{12}{n(n+1)} \sum_{j=1}^{k} \frac{R_j^2}{n_1} - 3(n+1)$$

k= number of groups

n_j= number of observations in the j^{th} group

n= number of observations in all groups combined

$R_{j=}$ sum of ranks in the j^{th} group

P value

The **P value** or calculated probability was the estimated probability of rejecting the null hypothesis (H0) of a study question when that hypothesis was true. The smaller the p-value, the more significant the result was said to be. All *P*-values are two tailed, and confidence intervals were calculated at the 95% level. Differences between the two populations were considered significant when $p \leq 0.05$.

RESULTS

The study compared the use of bone graft (DMBM) alone and in combination with platelet rich fibrin (PRF) in treatment of human periodontal intrabony defects.

A total of 18 sites in 15 patients within the age group of 25-40 years were selected for the study. However, 2 sites in each group could not be evaluated after surgical management since 2 became medically compromised and 2 were lost during follow up period, for reasons unrelated to the study.

The final results and statistical analysis was done for a total of 14 sites, 7 sites in each group.

Group I – 7 sites were treated with open flap debridement followed by placement of bone graft (DMBM).

Group II - 7 sites were treated with open flap debridement followed by placement of combination of bone graft (DMBM) and platelet rich fibrin (PRF).

All patients showed good compliance and healing period was uneventful for both the groups, without any signs of infections and complications, indicating biocompatibility of both grafting modalities.

The observations and results of various parameters are summarized in the tables and figures. Clinical parameters for both the groups are listed in tables 1 and 5 for their master chart observations and mean ± SD values respectively. Radiographic parameters are listed in tables 2, 3, 4 for their master chart observations, correction factor values and radiographic parameters respectively.

Figures 5,6,7,8 and figures 9, 10,11,12,13 diagrammatically represent clinical and radiographic parameters respectively in both the groups.

CLINICAL PARAMETERS

1. **Plaque Index** (*Table 5, Figure 4)*

Intragroup comparison

Group I : The mean plaque index score at baseline was 0.69±.19 and at 6 months was a 0.50± 0.20. The mean reduction in plaque index from baseline to 6 months was 0.19 which was moderately statistically significant (p=0.059).

Group II : The mean plaque index score at baseline was 0.61± 0.20 and at 6 months was a 0.42± 0.24. The mean reduction in plaque index from baseline to 6 months was 0.18 which was moderately statistically significant (p=0.059).

Intergroup comparison

Mean difference between group I and group II was 0.08 at baseline and 0.08 at 6 months which were statistically non-significant (p=0.71, p=0.54 respectively).

2. **Gingival Bleeding Index** *(Table5, Figure 5)*

Intragroup comparison

Group I : The mean gingival bleeding index score at baseline was 1.00±0.00 and at 6 months was a 0.29 ± 0.49. The mean reduction in gingival bleeding index from baseline to 6 months was 0.71 which was statistically significant (p=0.025).

Group II : The mean gingival bleeding index score at baseline was 1.00±0.00 and at 6 months was a 0.14± 0.38. The mean reduction in gingival bleeding index from baseline to 6 months was 0.86 which was statistically significant (p=0.014).

Intergroup comparison

Mean difference between group I and group II at 6 months was 0.15 which was statistically non-significant (p=0.71).

3. Probing pocket depth (*Table 5, Figure 6)*

Intragroup comparison

Group I : The mean pocket depth at baseline was 7.14 ± 0.69 and at 6 months was a 3.00 ± 1.53. The mean reduction in pocket depth from baseline to 6 months was 4.14 which was statistically significant (p=0.017).

Group II : The mean pocket depth at baseline was 7.43 ±1.51 and at 6 months was a 3.00 ± 1.53. The mean reduction in pocket depth from baseline to 6 months was 4.43 which was statistically significant (p=0.018).

Intergroup comparison

The mean difference in pocket depth between group I and group II at baseline was 0.28 and at 6 months was 0.29 which were statistically non-significant (p=0.710, p=1.00 respectively).

4. Clinical Attachment level (*Table 5, Figure 7)*

Intragroup comparison

Group I : The mean attachment level at baseline was 7.86 ±0.69 and at 6 months was a 4.57 ± 1.13. The mean gain in attachment level from baseline to 6 months was 3.29 which was statistically significant (p=0.017).

Group II : The mean attachment level at baseline was 8.29 ± 1.98 and at 6 months was a 3.86 ± 1.46. The mean reduction in attachment level from baseline to 6 months was 4.43 which was statistically significant (p=0.018).

Intergroup comparison

Mean difference in attachment level between group I and group II at baseline was 0.43 and at 6 months was 0.71 which were statistically non-significant (p=0.54, p=0.38 respectively).

RADIOGRAPHIC PARAMETERS

1. **Defect depth** (*Table 6, Figure 8)*

Intragroup comparison

Group I :

The mean defect depth at baseline was 4.89 ± 1.59 , at 3 months was 3.39 ± 1.23 and at 6 months was a 2.79 ± 1.15.

The mean difference in defect depth from baseline to 3 months was 1.50 which was statistically significant (p = 0.18) .The mean difference in defect depth from baseline to 6 months was 2.10 which was also statistically significant (P=0.18).The mean difference in defect depth from 3 months to 6 months was 0.60 which was statistically non-significant (p=0.176)

Group II :

The mean defect depth at baseline was 4.79 ± 3.15 , at 3 months was 2.26 ± 1.23 and at 6 months was a 1.31 ± 0.67.

The mean difference in defect depth from baseline to 3 months was 2.53 which was statistically significant (p = 0.18) .The mean difference in defect depth from baseline to 6 months was 3.48 which was also statistically significant (P=0.18). The mean difference in defect depth from 3 months to 6 months was 0.95 which was statistically non-significant (p=0.128).

Intergroup comparison

At 3 months mean difference in defect depth between group I and group II was 1.13 which was statistically not significant (p=0.073).

At 6 months mean difference in defect depth between group I and group II was 1.48 which was statistically significant (p=0.007).

2. Bone Fill *(Table 6)*

Intragroup comparison

Group I :

The mean bone fill at 3 months was 1.47 ± 1.26 and at 6 months was a 2.52 ± 1.30. The mean difference in bone fill from 3 months to 6 months was 1.05 which was statistically significant (p = 0.028).

Group II :

The mean bone fill at 3 months was 2.73 ± 1.92 and at 6 months was a 4.28 ± 2.64.

The mean difference in bone fill from 3 months to 6 months was 1.55 which was statistically significant (p = 0.028).

Intergroup comparison

At 3 months mean difference in bone fill between group I and group II was 1.26 which was statistically not significant (p=0.383).

At 6 months mean difference in defect depth between group I and group II was 1.76 which was statistically not significant (p=0.259).

3. Bone Fill % *(Table 6, Figure 9)*

Intragroup comparison

Group I :

The mean bone fill percentage at 3 months was 15.14 ± 11.76 and at 6 months was a 33.04 ± 21.01. The mean difference in bone fill from 3 months to 6 months was 17.80 which was statistically significant (p = 0.018).

Group II :

The mean bone fill at 3 months was 24.86 ± 14.18 and at 6 months was a 39.00 ± 12.89. The mean difference in bone fill percentage from 3 months to 6 months was 14.14 which was statistically significant (p = 0.028).

Intergroup comparison

At 3 months mean difference in bone fill percentage between group I and group II was 9.72 which was statistically not significant (p=0.318).

At 6 months mean difference in bone fill percentage between group I and group II was 5.96 which was statistically not significant (p=0.259).

4. Bone crest change *(Table 6)*

Intragroup comparison

Group I :

The mean change in bone crest at 3 months was 0.131 ± 0.73 and at 6 months was a 0.607 ± 0.50. The mean difference in change in bone crest from 3 months to 6 months was 0.476 which was statistically not significant (p = 0.128).

Group II :

The mean change in bone crest at 3 months was 0.384 ± 0.85 and at 6 months was a 0.725 ± 1.18. The mean difference in change in bone crest from 3 months to 6 months was 0.341 which was statistically not significant (p = 0..499).

Intergroup comparison

At 3 months mean difference in bone crest level between group I and group II was 0.253 which was statistically not significant (p=0.456).

At 6 months mean difference in bone crest level between group I and group II was 0.118 which was statistically not significant (p=0.620).

5. Bone Crest change % (*Table 6, Figure 10)*

Intragroup comparison

Group I :

The mean percentage change in bone crest at 3 months was 2.82 ± 18.22 and at 6 months was a 14.03 ± 10.58. The mean difference in percentage change in bone crest from 3 months to 6 months was 11.21 which was statistically not significant (p = 0.237).

Group II :

The mean percentage change in bone crest at 3 months was 2.90 ± 22.40 and at 6 months was 8.49 ± 20.03. The mean difference in percentage change in bone crest from 3 months to 6 months was 5.59 which was statistically not significant (p = 0.398).

Intergroup comparison

At 3 months mean percentage difference in bone crest level between group I and group II was 0.08 which was statistically not significant (p=0.805).

At 6 months mean percentage difference in bone crest level between group I and group II was 5.54 which was statistically not significant (p=0.620).

6. Defect resolution % (*Table 6, Figure 11)*

Group I :

The mean percentage defect resolution at 3 months was 12.43 ± 14.14 and at 6 months was a 36.92 ± 29.12. The mean difference in percentage defect resolution from 3 months to 6 months was 24.49 which was statistically not significant (p = 0.063).

Group II :

The mean percentage defect resolution at 3 months was 40.23 ± 29.41 and at 6 months was 59.52 ± 28.90. The mean difference in percentage defect resolution from 3 months to 6 months was 19.29 which was statistically not significant (p = 0.176).

Intergroup comparison

At 3 months mean percentage defect resolution between group I and group II was 27.60 which was statistically significant (p=0.038).

At 6 months mean percentage defect resolution between group I and group II was 22.60 which was statistically not significant (p=0.209).

Table 1 **: Master Chart I – CLINICAL PARAMETERS**

	Group I (DMBM)									
S.No	**Age (years)**	**Sex (M/F)**	**PI (Baseline)**	**GBI (Baseline)**	**PPD (mm) (Baseline)**	**CAL(mm) (Baseline)**	**PI 6 months**	**GBI 6 months**	**PPD (mm) 6 months**	**CAL (mm) 6 months**
1	43	M	1	1	7	8	0.5	0	2	6
2	28	F	0.5	1	7	7	0.25	0	5	5
3	29	F	0.75	1	8	8	0.5	0	3	3
4	44	F	0.75	1	7	7	0.75	1	5	5
5	35	M	0.50	1	8	8	0.25	0	3	3
6	26	F	0.75	1	7	9	0.75	1	1	5
7	24	F	0.5	1	6	8	0.5	0	2	5
	Group II (PRF-DMBM)									
	Age (years)	**Sex (M/F)**	**PI (Baseline)**	**GBI (Baseline)**	**PPD (mm) (Baseline)**	**CAL(mm) (Baseline)**	**PI 6 months**	**GBI 6 months**	**PPD (mm) 6 months**	**CAL(mm) 6 months**
1	24	M	0.75	1	8	8	0.75	0	3	3
2	43	M	0.5	1	7	10	0.25	0	1	5
3	26	F	0.75	1	5	5	0.25	0	2	2
4	40	F	0.5	1	10	11	0.25	0	3	5
5	29	F	0.75	1	7	7	0.75	1	5	5
6	39	M	0.25	1	8	8	0.25	0	5	5
7	39	M	0.75	1	7	9	0.5	0	2	2

Table 2 : **Master Chart II - RADIOGRAPHIC MEASUREMENTS**

Group I (DMBM)

	Baseline		After 3 months			After 6 months		
S.No	CEJ-BD	CEJ-AC	CEJ-BD	CEJ-AC	Correction factor CF_3	CEJ-BD	CEJ-AC	Correction factor CF_6
1	10.95	3.89	7.66	4.41	1.04	6.71	4.40	1.00
2	8.35	5.84	7.75	5.73	0.99	6.35	4.26	1.07
3	9.76	4.42	6.68	2.86	1.03	5.57	2.93	1.14
4	6.81	2.87	5.89	3.34	1.02	6.67	3.64	0.94
5	10.46	6.87	10.01	6.83	1.03	8.3	7.05	0.97
6	9.60	3.65	8.88	3.08	1.00	7.79	2.80	1.00
7	10.20	4.38	8.24	5.37	0.98	7.32	4.47	0.99

Group II (PRF-DMBM)

	Baseline		After 3 months			After 6 months		
S.No	CEJ-BD	CEJ-AC	CEJ-BD	CEJ-AC	Correction factor CF_3	CEJ-BD	CEJ-AC	Correction factor CF_6
1	13.79	4.44	7.05	4.96	1.03	5.53	4.52	1.06
2	10.63	7.71	8.65	6.96	1.01	7.45	4.80	1.03
3	5.77	2.14	5.47	2.92	1.03	4.20	2.64	0.97
4	14.90	5.58	11.11	5.68	1.07	6.73	5.68	1.03
5	8.13	6.01	6.85	5.86	0.86	6.05	5.17	1.02
6	8.04	5.54	5.17	3.96	1.00	4.80	4.1	0.99
7	10.42	6.7	8.26	6.74	0.97	7.60	6.25	0.89

Measurements in millimetres (mm)

Table 3 : **Correction factor (CF) calculation for radiographic parameters**

Group I (DMBM)

	CEJ-RA (Baseline)	**CEJ-RA (3 months)**	**CEJ-RA (6 months)**	**Correction Factor (3 months)**	**Correction Factor (6 months)**
1	18.77	18.10	18.79	1.04	1.00
2	13.63	13.71	12.71	0.99	1.07
3	15.19	14.76	13.27	1.03	1.14
4	11.39	11.16	12.09	1.02	0.94
5	16.38	15.95	16.96	1.03	0.97
6	13.60	13.62	13.55	1.00	1.00
7	13.02	13.24	13.19	0.98	0.99

Group II (PRF-DMBM)

	CEJ-RA (Baseline)	**CEJ-RA (3 months)**	**CEJ-RA (6 months)**	**Correction Factor (3 months)**	**Correction Factor (6 months)**
1	15.09	14.61	14.18	1.03	1.06
2	17.37	17.18	16.86	1.01	1.03
3	14.69	14.17	15.10	1.03	0.97
4	21.92	20.40	21.27	1.07	1.03
5	12.36	14.35	12.10	0.86	1.02
6	10.46	10.42	10.55	1.00	0.99
7	17.25	17.79	19.49	0.97	0.89

Measurements in millimetres (mm)

Table 4: **Master Chart III – RADIOGRAPHIC PARAMETERS**

	Group I (DMBM)												
S.no	**DD -BL**	**DD 3**	**DD 6**	**BF 3**	**BF 6**	**BF % 3**	**BF % 6**	**BCC 3**	**BCC 6**	**BCC % 3**	**BCC % 6**	**DR % 3**	**DR % 6**
1	7.06	3.38	2.31	3.06	4.42	27.9	40.36	-0.7	0.51	-17.99	13.11	33.43	55.38
2	2.51	1.99	2.21	0.68	1.55	8.1	18.56	0.17	1.28	2.91	21.91	20.31	10.75
3	5.34	3.93	3.01	2.88	3.41	29.5	77.15	1.47	1.08	33.26	24.43	26.4	43.63
4	3.94	2.6	2.99	0.8	0.54	11.75	18.82	-0.54	0.55	-18.81	16.08	6.59	0.25
5	3.59	3.27	1.21	-0.16	2.99	-1.53	28.59	-0.16	0.03	2.32	0.44	0	82.45
6	5.95	5.8	4.99	0.72	1.81	7.5	18.85	0.57	0.85	15.6	23.29	2.5	16.13
7	5.82	2.81	2.82	2.32	2.95	22.76	28.96	0.11	-0.05	2.45	-1.03	-2.23	49.82
	Group II (PRF-DMBM)												
	DD 0	**DD 3**	**DD 6**	**BF 3**	**BF 6**	**BF % 3**	**BF % 6**	**BCC 3**	**BCC 6**	**BCC % 3**	**BCC % 6**	**DR % 3**	**DR % 6**
1	9.35	2.15	1.07	6.52	7.94	47.28	57.58	0.67	-0.35	15.9	-7.88	62.56	81.18
2	2.91	1.71	2.73	1.89	2.96	17.78	27.84	0.68	2.77	8.82	35.93	41.58	6.53
3	3.63	2.62	1.51	0.14	1.7	2.42	29.39	-0.87	-0.42	-40.5	-19.63	-20.11	35.26
4	9.32	5.81	1.08	3.01	7.97	20.22	53.45	-0.5	-0.27	-8.92	-4.84	26.93	82.62
5	2.12	0.85	0.9	2.24	1.96	27.55	24.11	0.97	0.73	14.16	12.15	59.9	58.01
6	2.5	1.21	0.69	2.87	3.29	35.7	40.92	1.58	1.48	28.52	26.71	51.6	72.4
7	3.72	1.47	1.2	2.41	4.14	23.13	39.73	0.16	1.14	2.34	17.01	59.14	80.65

Measurements in millimetres (mm)

Table 5 **: COMPARISON OF CLINICAL PARAMETERS – GROUP I AND GROUP II**

	Group I			**Group II**			**Group I v/s Group II**			
	At baseline	**At 6 months**		**At baseline**	**At 6 months**		**At baseline**		**At 6 months**	
	Mean ±SD	Mean ±SD	**p value**	Mean ±SD	Mean ±SD	**p value**	Mean diff	**p value**	Mean diff	**p value**
PI	0.69 ± 0.19	0.50 ± 0.20	**0.059**#	0.61 ± 0.20	0.42 ± 0.24	**0.059**#	0.08	0 .71	0.08	0.54
GBI	1.00 ± 0.00	0.29 ± 0.49	**0.025***	1.00 ± 0.00	0.14 ± 0.38	**0.014***	0.00	1.00	0.15	0.71
PPD (mm)	7.14 ± 0.69	3.00 ± 1.53	**0.017***	7.43 ± 1.51	3.00 ± 1.53	**0.018***	0.29	0.71	0.00	1
CAL (mm)	7.86 ± 0.69	4.57 ± 1.13	**0.017***	8.29 ± 1.98	3.86 ± 1.46	**0.018***	0.43	0.54	0.71	0.38

Wilcoxon Signed ranks test for Intragroup comarisons
Mann-Whitney U test for Intergroup comparisons

***Significant**
 # Moderately Significant

Table 6 : **COMPARISON OF RADIOGRAPHIC PARAMETERS – GROUP I AND GROUP II**

	Group I				**Group II**				**Group I v/s Group II**			
	At baseline	**At 3 months**	**At 6 months**		**At baseline**	**At 3 months**	**At 6 months**		**At 3 months**		**At 6 months**	
	Mean±SD	Mean±SD	Mean±SD	**p value**	Mean±SD	Mean±SD	Mean±SD	**p value**	Mean diff	**p value**	Mean diff	**p value**
Defect depth (mm)	4.89 ± 4.59	3.39 ± 1.23	2.79 ± 1.15	**0.035***	4.79 ± 3.15	2.26 ± 1.23	1.31 ± 0.67	**0.007***	1.13	0.073	1.48	**0.007***
Bone fill (mm)	-	1.47 ± 1.26	2.52 ± 1.30	**0.028***	-	2.73 ± 1.92	4.28 ± 2.64	**0.028***	1.26	0.383	1.76	0.259
Bone fill %	-	15.14 ± 11.76	33.04 ± 21.01	**0.018***	-	24.86 ± 14.18	39.00 ± 12.89	**0.028***	9.72	0.318	5.96	0.259
Bone crest change (mm)	-	0.131 ± 0.73	0.607 ± 0.50	0.128	-	0.384 ± 0.85	0.725 ± 1.18	0.499	0.253	0.456	0.118	0.620
Bone crest change %	-	2.82 ± 18.22	14.03 ± 10.58	0.237	-	2.90 ± 22.40	8.49 ± 20.03	0.398	0.08	0.805	5.54	0.620
Defect Resolution %	-	12.43 ± 14.14	36.92 ± 29.12	0.063	-	40.23 ± 29.41	59.52 ± 28.90	0.176	27.60	**0.038***	22.60	0.209

Wilcoxon Signed ranks test for Intragroup comparisons
Mann-Whitney U test for Intergroup comparisons
Kruskal-Wallis test for the multivariate analysis in individual groups

***Significant**
 # Moderately Significant

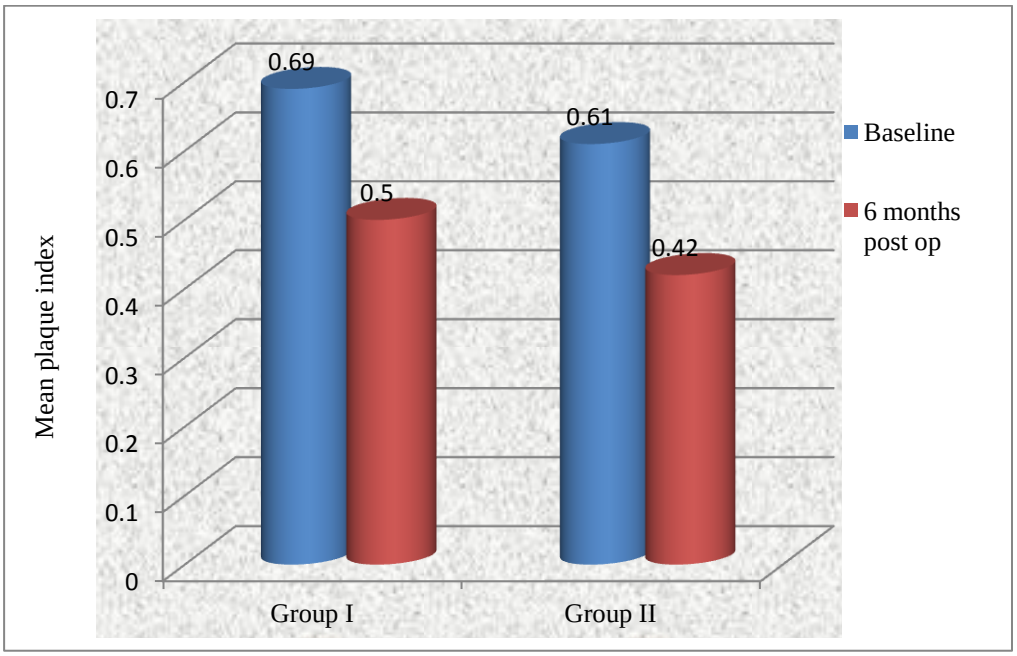


Figure 4 : Comparison of Plaque Index (PI) between group I and group II

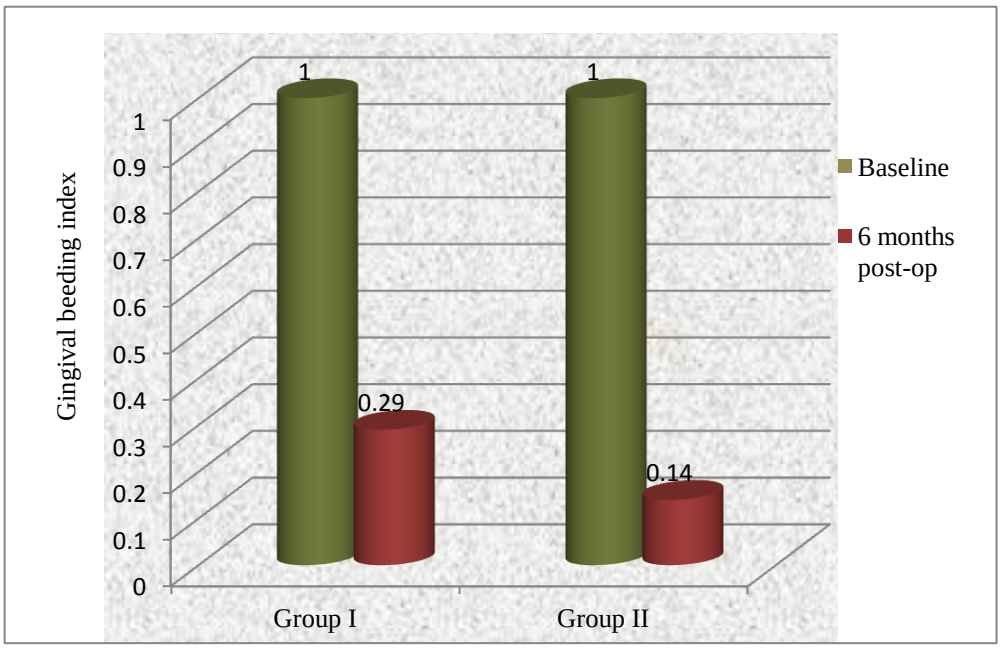


Figure 5 : Comparison of Gingival Bleeding Index (GBI) between group I and group II

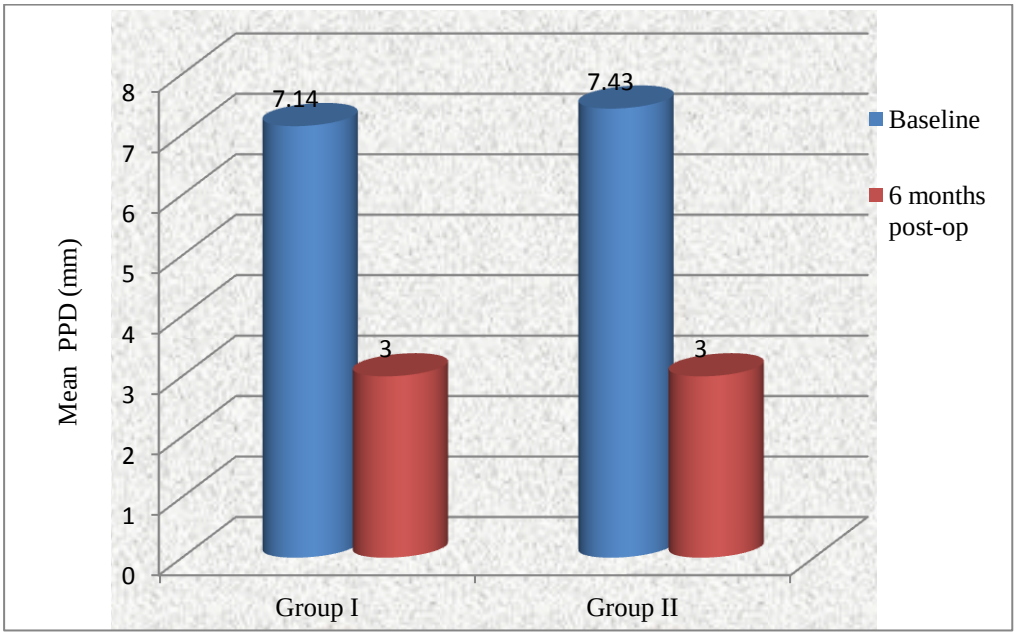


Figure 6 : Comparison of Probing pocket depth (PPD) between group I and group II

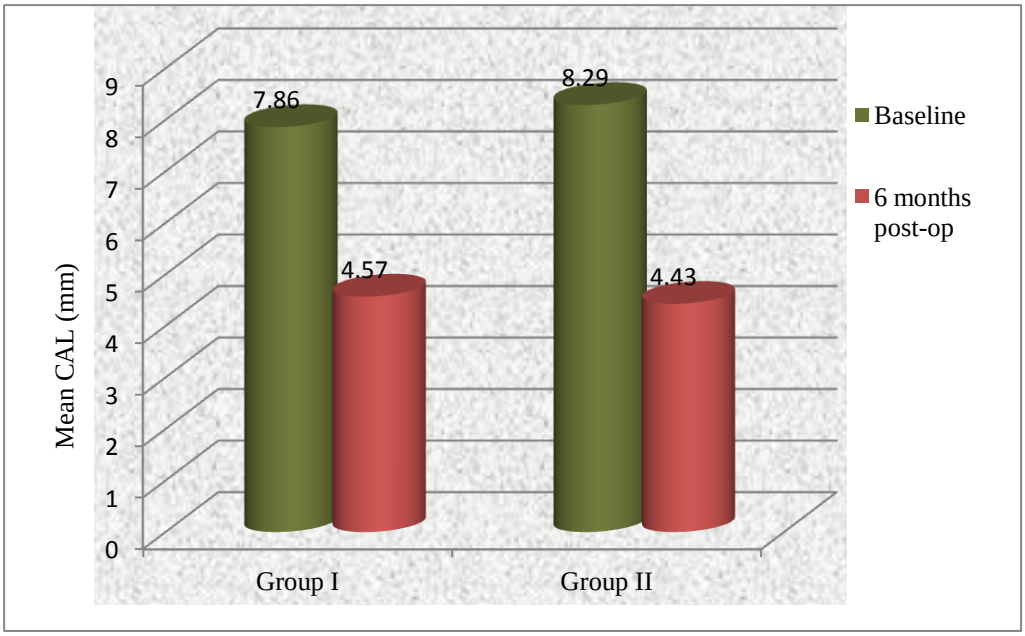


Figure 7 : Comparison of Clinical attachment level (CAL) between group I and group II

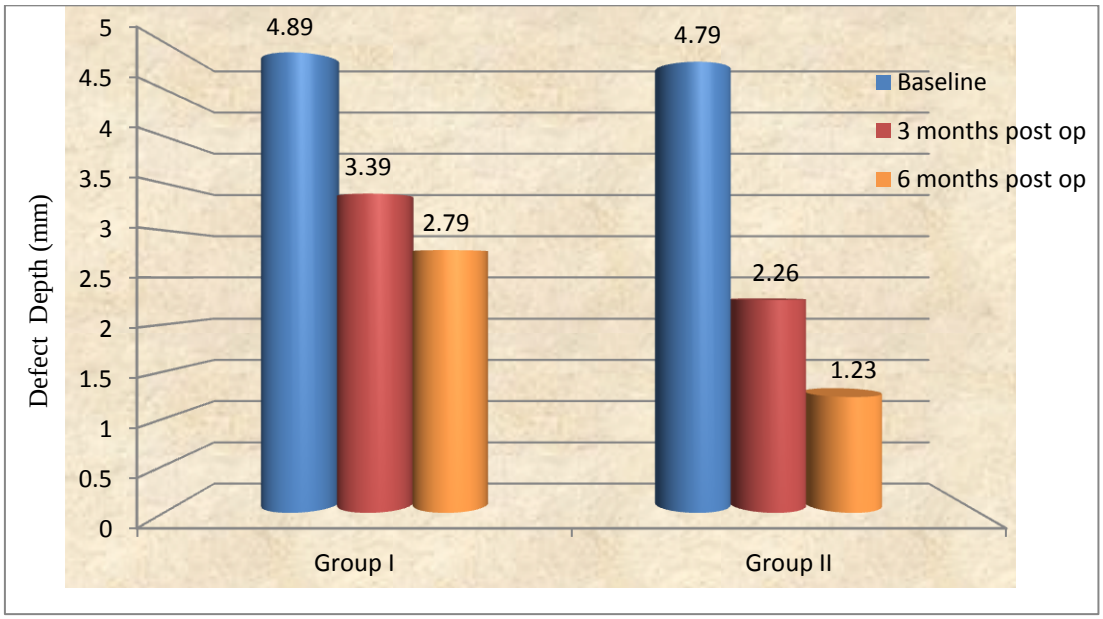


Figure 8 : Comparison of Defect Depth (mm) between group I and group II

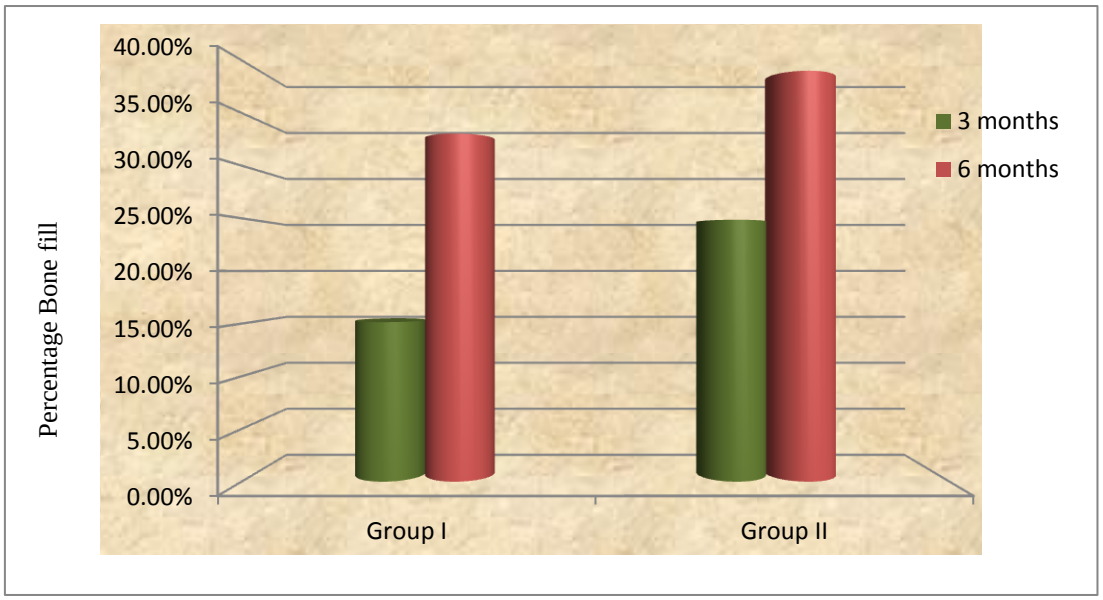


Figure 9 : Comparison of percentage of Bone fill between group I and group II

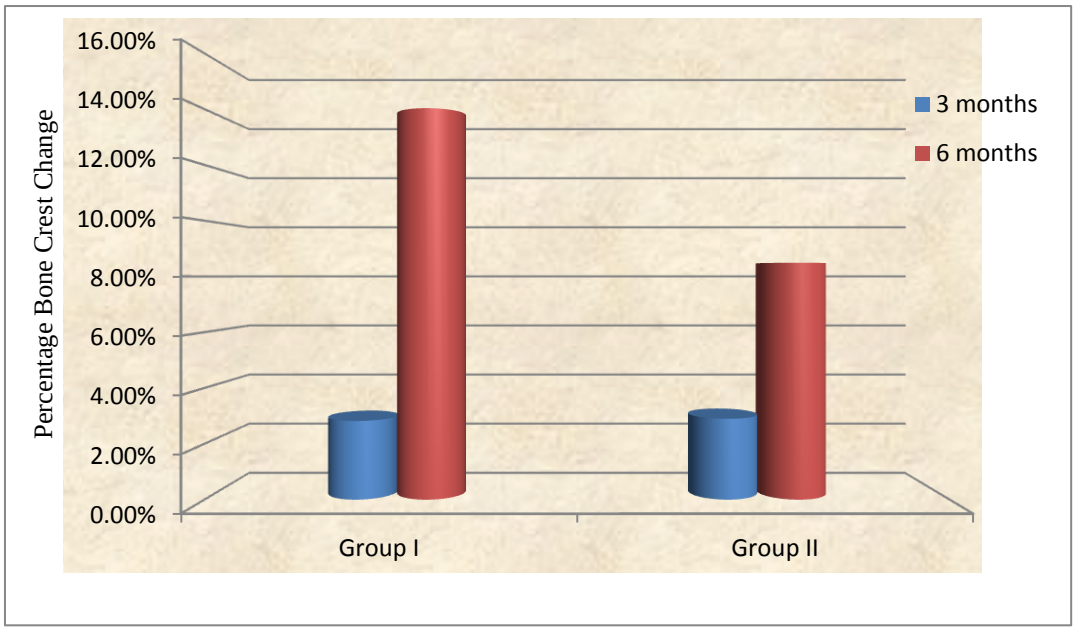


Figure 10 : Comparison of percentage of Bone crest change between group I and group II

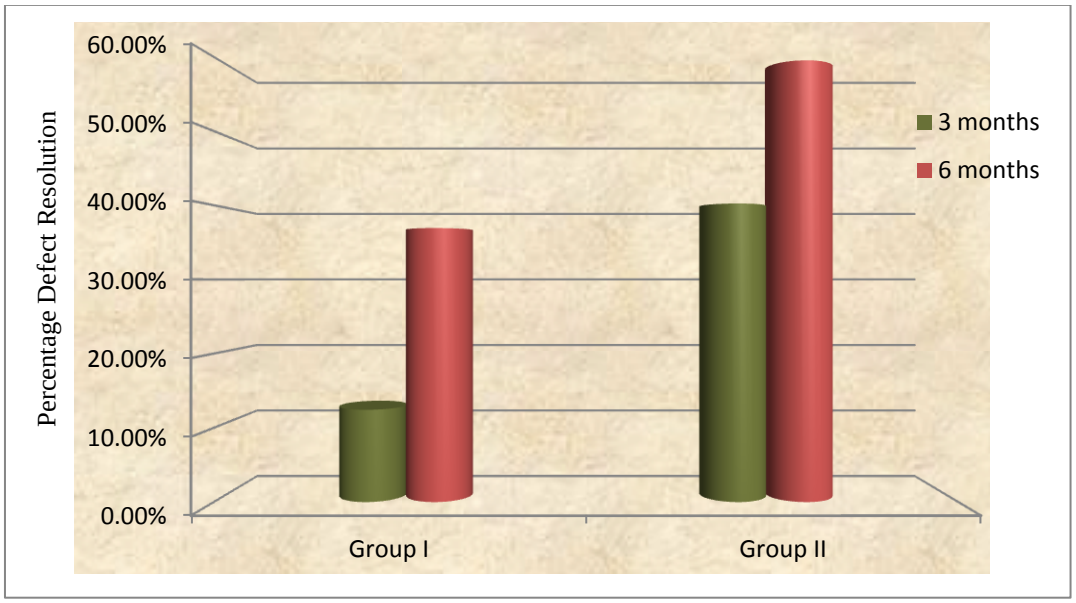


Figure 11 : Comparison of percentage of Defect Resolution between group I and group II

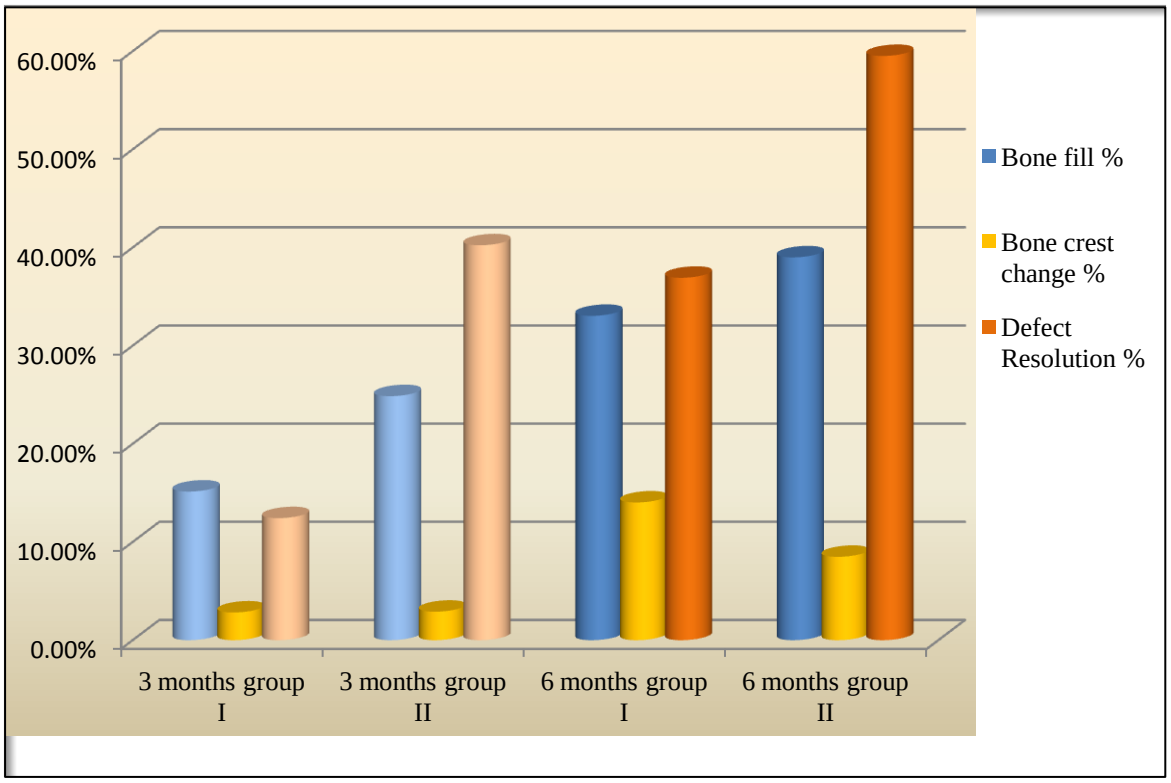


Figure 12 : Comparison of Radiographic Parameters

DISCUSSION

Periodontal therapy is performed with the primary objectives of gaining access to the diseased sites, achieving reduction in pocket depth, arresting further disease progression and finally restoring the periodontal tissues lost due to disease process.

The ultimate aim to achieve periodontal regeneration via new attachment formation has been approached by variety by regenerative modalities, but none has been established as a gold standard, given their own associated limitations.

The recent trend of endogenous replacement therapy has shifted the focus to application of autologous mitogenic proteins to periodontal wound. One such store house of autologous growth factors and leukocytes, recently made available to the field of periodontal therapy, is Platelet Rich Fibrin.

Presently, researchers are in the process of exploring the vast benefits of PRF that can revolutionalize the field of periodontal regeneration. However, till date very few clinical trials have been attempted on application of PRF alone and in combination with bone grafts in management of periodontal intrabony defects.

Thus, given the limited available literature on PRF in combination with bone grafts, this study was planned and undertaken.

The decision to utilize PRF was made given its advantageous properties, inexpensive nature, ease of manipulation and delivery to surgical site. PRF has a tendency to get resorbed in approximately 7-10 days.[30] Hence, DMBM was added hypothesizing that it could enhance the effects of PRF by its osteoinductive and osteoconductive properties, by maintaining space at the wound site and allowing guided tissues regeneration to occur.

For this purpose, a total of 18 sites in 15 patients were taken up for study, of which 4 patients could not be evaluated due to reasons unrelated to the study. Thus a

total of 14 sites in 12 patients were evaluated, i.e. 7 sites per group. Patients from age group 25-40 years with moderate periodontitis were included in the study. This was in accordance with ***Deas and Mealey's***[25] inclusion criteria, that if risk factor especially smoking can be eliminated and compliance with maintenance care is high, then surgical regenerative therapy can be as beneficial to the patients with aggressive periodontitis as to chronic periodontitis.

Care was taken to include intrabony defects with three or combined wall defects only, as they provide the best spatial relationship for defect bridging by vascular and cellular elements from PDL and adjacent osseous walls. Also the presence of more number of defect walls provide space maintenance, protection and retention of grafts.[11]

On evaluation of clinical parameters, plaque index showed similar clinical values in both groups before and after the treatment, with no significant change thus suggesting good hygiene maintenance by all patients during the course of the study. These results coincide with those of studies by ***Yukna*** *et al*[108] and ***Srikanth*** *et al*[97] who observed that patients undergoing periodontal therapy try to maintain optimal oral hygiene.

According to ***Rosen et al***[85], periodontal probing and recording of attachment levels should not be done for atleast 6 months post-surgically, since probing can damage the healing site, thereby diminishing regenerative outcomes. Thus, in the present study probing related clinical parameters were recorded at baseline and 6 months post surgically only.

The mean pocket depths in both the DMBM and PRF – DMBM groups at baseline were 7.14mm and 7.43mm respectively, signifying that cases with similar severity of defects were selected for both the groups. Significant reductions were

observed in pocket depths of both the groups (4.14 mm in group I and 4.43mm in group II) at the end of 6 months. This improvement in combination group was in accordance with ***Lekovic's*** observation of 4.47 mm in their PRF-BPBM group[62].

Significant gain in clinical attachment levels were also observed in both the groups at the end of 6 months. However, better levels were observed with PRF – DMBM combination (4.43 ± 1.81 mm) than with DMBM alone (3.29 ± 1.38 mm). The gain was even better than PRF-BPBM group of the comparative study by ***Lekovic et al*** (3.82 ± 6.78).[62]

Radiographic assessments were done at baseline, at the end of 3 months and at the end of 6 months using consequent intra-oral radiographs. While using radiographs in periodontal diagnosis and research technical and geometric variables need to be considered. Projection geometry and parameters should be standardized to minimize measurement errors in serial radiography ***(Lang & Hill, 1977)***[68]. Pre-fabricated film holders like that used in this study may provide projection standardization to a major degree.

As documented by ***Gupta et al***[45], radiographic evidence of bone changes can be observed as early as 3 months post-operatively. Also, it was hypothesized that PRF as a rich source of autologous growth factors and cytokines may lead to rapid changes in bone formation. Thus, keeping all of this in mind, radiographic evaluations for the present study were attempted at an early time period of 3 months.

On radiographic evaluation, progressively significant reduction in defect depth was observed in both the study groups at the end of 3 months and 6 months in comparison to baseline. Also, on comparing the two groups with each other significantly more reduction in defect depth was observed at the end of 6 months with

combination (PRF-DMBM) therapy. Thus, suggesting better regenerative potential of addition of PRF to bone graft DMBM.

After a follow-up period of 3 months, higher mean bone fill was observed in combination group (2.73 ± 1.92 mm) as compared to DMBM group (1.47 ± 1.26 mm) Also at 6 months, bone fill was more for PRF-DMBM combination group (4.28 ± 2.64 mm) than DMBM group (2.52 ± 1.30 mm). Moreover, the improvement in bone fill in combination group was much more at the end of 6 months which was in accordance with Lekovic's findings in PRF- BPBM group (4.06 ± 0.87 mm)[72].

In the present study, PRF-DMBM group showed mean percentage of bone fill to be upto 51.89% at the end of 6 months. However, ***Pradeep et al***[78] in their recently reported clinical trial in intrabony defects observed 69.39±16.52% of bone fill with PRF + HA combination at the end of 9 months. The variation in two studies may be attributed to the different follow-up periods and dissimilar graft materials used with PRF.

Change in the alveolar crest level is a frequent outcome of surgical periodontal therapy. The morphology of osseous defect and the type of therapy performed may have an influence on it. In the present study, the mean changes in the level of bone crest were statistically not significant. Although minimal in values, amount of change in alveolar crest determines the changes in dimensions of original defect.

Percentage of original defect resolution is an important parameter that takes into account not only the amount of bone fill but also the change in alveolar crest level, if any. In the present study, a significant change in defect resolution was observed as early as 3 months post-operatively. When compared to bone graft (DMBM) alone (12.43 %), the combination group showed much higher defect

resolution (40.23 %). This was a unique finding not observed in any of the PRF related studies, since an early radiographic analysis was not attempted by them.

Osseous changes as measured by intraoral radiographs may be different from the direct observations by surgical re-entry. This may be attributed to our inability to accurately determine the radiographic landmarks, obscuration of the deepest portion of the defect by facial or lingual cortices or tooth root and overestimation or underestimation of radiographic parameters.

In the present study, an appreciable amount of defect resolution was observed at 6 months for PRF-DMBM group (59.52 ± 28.90 %), which was much higher than DMBM group (36.92 ± 29.12 %). However, the difference between the two groups was statistically non-significant which can be attributed to the possible radiographic limitations.

The present study has shown good results and additional benefits in clinical and radiographic parameters when PRF was used in combination with DMBM. Thus in future, PRF may prove to be a novel adjunct to conventional regenerative therapeutic modalities in management of periodontal osseous defects.

SUMMARY AND CONCLUSION

In the present study, a total of 14 sites with periodontal intrabony defects were evaluated for management with demineralised bone matrix (DMBM- osseograft™) alone and in combination with platelet rich fibrin. The aim of the study was to evaluate the clinical effectiveness and regenerative potential of both the grafting modalities and to explore the additional effectiveness of PRF on DMBM, if any.

After the collection of clinical and radiographic data over a period of 6 months, the values were subjected to statistical analysis and the following conclusions were drawn :

1. Both the graft materials, DMBM and PRF were well tolerated by the periodontal tissues during the course of the study.
2. Clinical parameters demonstrated significant improvement in both the groups at the end of 6 months.
3. Radiographic evidence of defect depth reduction and bone fill was observed in relation to both groups. The difference at the end of 3 months and 6 months was statistically significant.
4. Addition of PRF revealed its beneficial effect in the combination group with evidence of significant defect resolution at an early follow up period of 3 months.

Outcomes of periodontal regenerative therapy, that is, its success and failure are dependent on multiple factors. Therefore, it is important for us to determine our therapeutic goals realistically. Overall parameters such as patient selection, defect selection, choice of diagnostic and therapeutic modalities and post operative follow up period, all should be taken well into consideration during decision making.

Within the limits of present study, addition of Platelet Rich Fibrin to bone graft demonstrated successful and promising results. Thus in future, clinical trials with larger sample size may be employed to further explore the potential of PRF as a grafting modality.

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Annexure 1: Information Sheet

INFORMATION SHEET

- We are conducting a study on **"** Evaluation of clinical effectiveness of Platelet Rich Fibrin and bone graft in management of intrabony defects" among patients attending TNGDCH, Chennai and for this study we are selecting patients.

- The privacy of patients in research will be maintained throughout the study. In event of any publication or presentation resulting from the research, no personally identifiable information will be shared

- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time. Your decision will not result in any loss or benefits to which you are otherwise entitled.

- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in management or treatment

Annexure 2: Informed Consent Form - English

STUDY TITLE:

"Evaluation of clinical effectiveness of platelet rich fibrin and bone graft in management of intrabony defects : A COMPARATIVE STUDY",

Name: O.P.No:

Address: S. No: Group no:

Age / Sex:
Tel. no:

I, _____ age _____ years exercising my free power of choice, hereby give my consent to be included as a participant in the study **"Evaluation of clinical effectiveness of Platelet Rich Fibrin and bone graft in management of intrabony defects: A Comparative study**"

I agree to the following:

- I have been informed to my satisfaction about the purpose of the study and study procedures including investigations to monitor and safeguard my body function.
- I understand that the lab investigations will require the procurement of my blood in required amount.
- I agree to undergo the surgical procedure involved in the study process.
- I agree to cooperate fully and to inform my doctor immediately if I suffer any unusual symptom.
- I have informed the doctor about all medications I have taken in the recent past and those I am currently taking.
- I hereby give permission to use my medical records for research purpose. I am told that the investigating doctor and institution will keep my identity confidential.
- I am willing for the bone graft placement, knowing the same has been procured from healthy donors.

Name of the patient Signature / Thumb impression

Name of the investigator Signature Date

Annexure 3: Tamil Consent Form

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு

பிளேட்லெட் ரிச் ஃபைபரின் மற்றும் மாற்று எலும்பு துகள்கள் கொண்டு எலும்பின் தேய்மானத்தை தீர்வு செய்ய ஒப்பிட்டு நோக்கும் ஆய்வு

பெயர்: புறநோயாளி எண்:

முகவரி: ஆராய்ச்சி சேர்க்கை எண்:

வயது: பாலினம்: ஆண்: பெண்

நான் ..... வயது ..... என்னுடைய சுயநினைவுடனும் மற்றும் முழு சுதந்திரத்துடனும் என்னை இம்மருத்துவ ஆராய்ச்சியில் சேர்த்துக் கொள்ள ஒப்புதல் அளிக்கிறேன்.

கீழ்காணப்படும் நிபந்தனைகளுக்கு நான் ஒப்புதல் அளிக்கிறேன்

- இந்த ஆராய்ச்சியின் நோக்கமும் மற்றும் செயல் முறைகளும் (என்னுடைய உடல் நிலையை பாதுகாக்கவும் அதை கண்காணிக்கவுமான பரிசோதனைகள் உட்பட எனக்கு திருப்தியளிக்கும் வகையில் என்னிடம் அறிவுறுத்தப்பட்டது.
- இந்த பரிசோதனை செய்வதற்காக புறத்திசுக்களில் அறுவை சிகிச்சை செய்ய வேண்டுயுள்ளதாக அறிகிறேன்.
- அறுவை சிகிச்சையின் போது எலும்புத்துகள்கள் வைக்க சம்மதிக்கிறேன்.
- என் உடல் நலம் பாதிக்கப்பட்டாலோ அல்லது எதிர்பாராத வழக்கத்திற்கு மாறான நோய்க்குறி தென்பட்டாலோ அதனை விலக்குவதற்கும் முழு உரிமை இருப்பதாகவும் அறிகிறேன்.
- நான் ஏற்கனவே உட்கொண்ட மற்றும் உட்கொள்கிற மருத்துகளைப் பற்றிய விபரங்கள் ஆராய்ச்சியாளரிடம் தெரிவித்துள்ளேன்.
- என் மருத்துவ குறிப்பேடுகளை இந்த ஆராய்ச்சியில் பயன்படுத்திக்கொள்ள சம்மதிக்கிறேன். இந்த ஆராய்ச்சி மையமும் ஆராய்ச்சியாளரும் என்னுடைய விவரங்கள் அனைத்தையும் இரகசியமாக வைப்பதாக அறிகிறேன்.

நோயாளியின் பெயர்	கையொப்பம்	தேதி
ஆராய்ச்சியாளரின் பெயர்	கையொப்பம்	தேதி

Annexure 4: Proforma

DEPARTMENT OF PERIODONTICS
TAMILNADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL
CHENNAI – 600003

"Evaluation of clinical effectiveness of Platelet Rich Fibrin and bone graft in management of intrabony defects: A COMPARATIVE STUDY",

PROFORMA

Date:	O.P. No:	Group no:
Name:	Age / Sex:	Case no :
Address:	Tel. no	Mobile no:
	Occupation:	Income:

Chief Complaint :

History of presenting illness :

Past Medical History:

Past Dental History:

Clinical Examination:

PERIODONTAL EXAMINATION

1. PLAQUE INDEX – SILNESS AND LOE (1964)

18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**

Calculation:

Score: **Inference:**

2. GINGIVAL BLEEDING INDEX – AINAMO AND BAY (1975)

18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**

Calculation

Score: **Inference:**

3. PROBING DEPTH (PD) & CLINICAL ATTACHMENT LEVEL (CAL) (mm)

MAXILLARY: **Buccal**

CAL																
PPD																
	18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
PPD																
CAL																

Palatal

MANDIBULAR: **Lingual**

CAL																
PPD																
	48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
PPD																
CAL																

Labial

4. **INVESTIGATIONS:**

Blood investigations :

Radiographic evaluation -

Others :

5. **Diagnosis:**

6. **Prognosis:**

TREATMENT

1. EMERGENCY / PRELIMNARY PHASE

2. PHASE I

3. RE-EVALUATION (at 4-6 weeks after phase I)

P LAQUE INDEX – SILNESS AND LOE (1964)

18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**

Calculation:

Score: **Inference:**

GINGIVAL BLEEDING INDEX – AINAMO AND BAY (1975)

18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**

Score:

Inference:

PROBING DEPTH (PD) & CLINICAL ATTACHMENT LEVEL (CAL) (mm)

MAXILLARY: Buccal

CAL																
PPD																
	18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
PPD																
CAL																

Palatal

MANDIBULAR: Lingual

CAL																
PPD																
	48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
PPD																
CAL																

Labial

4. **CLINICAL SITE SELECTED FOR STUDY -**

5. **PHASE II (Surgical) :**

6. **PHASE III**

7. PHASE IV

RE-EVALUATION & SUMMARY

CLINICAL EVALUATION :

SL No	Indices	Baseline	Post -op	
			3months	6 months
1.	Plaque index *(Silness and Loe, 1964)*			
2.	Gingival bleeding index– (A*inamo and bay, 1975*)			

Sl. No.	Calculations	Baseline	Post-op	
			3 months	6months
1.	Pocket Probing depth (mm)			
2.	Gingival recession (mm)			
3.	Clinical Attachment level (mm)			

RADIOGRAPHIC EVALUATION :

Sl. No.	Calculations	Baseline	Post op	
			3months	6months
1	CEJ to the base of the defect (mm)			
2	CEJ to the alveolar crest of the defect (mm)			

INFERENCE / RESULT

Signature of the P.G. Student **Signature of the Guide**

Date: